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## INVESTIGATIONS OF THE ROSETTE DISEASE OF WHEAT AND ITS CONTROL<sup>1</sup>

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### INTRODUCTION

It is the purpose of this paper to present the results obtained during the past three years in the investigations on the rosette disease of winter wheat. This disease was reported from Madison County, Ill., in the spring of 1919 by Lyman<sup>3</sup> and later it was found in other counties in Illinois and in several counties in Indiana. Shortly after its discovery it was described by Humphrey and Johnson (9).<sup>4</sup> At that time the disease was considered apparently identical with the take-all disease occurring in Australasia and the footrot disease occurring in Europe. However, investigations herein reported show that the rosette disease of wheat differs from take-all in plant symptoms and also in the varietal and host ranges. Furthermore, none of the fungi which are commonly found associated with take-all abroad and in parts of this country have been found associated with the rosette disease. It is therefore clearly evident that the latter disease occurring in Illinois and Indiana is distinctly different from the take-all disease.

The take-all, footrot, and similar diseases which occur abroad also occur in several States in this country.<sup>5</sup> While the causal agent may or may not be the same in all cases, the plant symptoms manifested in

<sup>1</sup> Accepted for publication Oct. 28, 1921. In this paper the designation rosette disease will be applied to the wheat disease occurring in Illinois and Indiana, previously referred to as take-all and so-called take-all. The name take-all will be used in referring to the disease caused by *Ophiobolus graminis* Sacc. The diseases producing symptoms more or less closely similar to take-all will be grouped under the name footrot. The investigations upon which this paper is based were carried on in cooperation with the Wisconsin, Illinois, and Purdue University Agricultural Experiment Stations, the Missouri Botanical Garden, and the Board of Education, Granite City, Ill.

<sup>2</sup> Especial credit is due Prof. G. H. Dungan, of the Illinois Agricultural Experiment Station, for bringing together certain fertilizers and seed samples used and for assisting in sowing some of the plots and in other ways, and Dr. C. E. Leighty, Agronomist, Office of Cereal Investigations, United States Department of Agriculture, for identifying wheat varieties and for furnishing all seed marked "C. I." in the tables. The writer is indebted to Prof. L. R. Jones and Dr. A. G. Johnson for helpful suggestions throughout the work herein reported.

<sup>3</sup> LYMAN, G. R. "TAKE-ALL" OF WHEAT IN THIS COUNTRY. U. S. Dept. Agr. Bur. Plant Indus. Plant Dis. Survey Circ. 1 p. May 1, 1919. Mimeographed.

<sup>4</sup> Reference is made by number (italic) to "Literature cited," pp. 799-800.

<sup>5</sup> A wheat disease of the take-all type was first reported in America from Oregon in 1902 by Cordley (3). None of the organisms which are associated with this type of trouble abroad were reported by him, however, and this type of disease was not reported again from Oregon until 1921 (16).

In 1902 the same type of wheat malady was reported from Roanoke County, Va. (20). Diseased plants sent to the Office of Cereal Investigations, United States Department of Agriculture, by Dr. F. D. Fromme on June 16, 1919, showed typical symptoms of take-all and footrot as they are described in the foreign literature. Examinations made by the office staff revealed the presence of mature perithecia of *Ophiobolus graminis* Sacc., the organism which is now known to cause take-all. Later this disease and organism were found in Washington (6), New York (14), Arkansas (26), Oregon (29), and Knox County, Ind. (26).

This type of malady associated with *Leptosphæria herpotrichoides* de Not has been found in Washington by the writer and with *Wormoscoia graminis* (McAlpine) Sacc. and D. Sacc. (21) in Kansas, Arkansas, and Oregon.

different localities have been observed to be very similar and in general they answer the descriptions for take-all and footrot as given in the foreign papers by Prillieux and Delacroix (26), Mangin (22), McAlpine (19), Foëx (4), and Waters (37).

The rosette disease of wheat was not reported until 1919; however, many farmers in the infested areas are certain that they have observed the trouble in wheat for many years. While such reports, of course, are not entirely conclusive, they indicate that the trouble may have been in existence in certain of the infested areas for a considerable length of time. The fact that the disease has recurred each year since it was reported in 1919 adds weight to this possibility. Furthermore, all attempts to establish its origin through channels of introduction have failed, indicating still further that the introduction of the disease has not occurred recently.

As early as 1802 a destructive wheat disease was reported by Hollingsworth (7, 8), and Mease (25)<sup>6</sup> from the district near Elkton, Md. In some ways the descriptions of this malady fit the symptoms for the rosette disease, while in other respects they seem to fit those for the true take-all. The descriptions of this trouble are not adequate, making it difficult or impossible to tell exactly whether or not it was the same as the rosette disease. Further light may be thrown on the matter of origin after the cause of the rosette disease has been determined.

#### COMMON NAME OF THE DISEASE

Shortly after the discovery of the disease in Illinois, the name take-all was applied to it by Humphrey and Johnson (9). Later, Stevens (33) designated the disease by the name footrot; and Johnson and Haskell (12) and McKinney (20)<sup>7</sup> have designated the trouble by the tentative name "so-called take-all." Since all of the evidence indicates that the disease is not the same as the Australian take-all, obviously the name take-all is not an appropriate one for it. With reference to the name footrot the American and the foreign literature shows that as a common name footrot is closely associated with the name take-all. This conception is confirmed further by plant pathologists from Australasia and Europe with whom the writer has conferred.<sup>8</sup> McAlpine (19), Massee (23), and Dana (2) consider these names to be practically synonymous. In view of this relationship, the writer prefers not to accept the name footrot for the Illinois disease. It, therefore, seems advisable to designate the disease by the more descriptive name "the rosette disease of wheat."

<sup>6</sup> These rather obscure references were brought to the attention of the writer through the kindness of Dr. G. H. Coons of the Michigan Agricultural Experiment Station.

<sup>7</sup> MCKINNEY, H. H. RESISTANCE IN WHEAT TO THE SO-CALLED TAKE-ALL DISEASE. In U. S. Dept. Agr. Bureau Plant Indus. Off. Cer. Inves. Cereal Courier, v. 12, no. 17, p. 229-231. 1920. Mimeographed.

<sup>8</sup> Dr. A. D. Cotton, Mycologist to the Board of Agriculture, Kew, England; Dr. E. T. Foëx, Director of Station of Vegetable Pathology, Paris; Dr. A. H. Cockayne, Government Biologist, New Zealand Department of Agriculture, Wellington, New Zealand; Dr. W. L. Waterhouse, University of Sydney, Sydney, New South Wales; W. R. Binks, Chatswood, New South Wales.

## DISTRIBUTION

So far as definitely known, the rosette disease of wheat occurs only in the States of Illinois and Indiana. Table I gives the counties in which the disease has been located and the number of fields and areas infested in each.

TABLE I.—*Distribution and severity of the rosette disease in the United States in 1919*

State.	County.	Number of fields infested.	Acreage in infested fields.	Extent of disease in infested fields.
Illinois.....	Madison.....	25	629	Trace to 85 per cent.
	Mason.....	48	1,310.5	Trace to 70 per cent.
	Sangamon.....	2	380	Trace to 5 per cent.
	Logan.....	1		
Indiana.....	Laporte.....	5	150	Trace.
	Porter.....	6	120	Trace to 63 per cent.
	Tippecanoe.....	1	5	Trace.

## ECONOMIC IMPORTANCE

While the occurrence of this trouble is restricted to relatively small areas, its importance must not be overlooked. Under ideal conditions for the disease it has caused as high as 40 per cent actual loss of grain in a 50-acre field. It is not uncommon to find many spots or large areas in a field where practically all the wheat plants are killed. Sometimes, however, the diseased plants in such spots recover to a considerable extent and produce some grain. However, such plants usually are so delayed in ripening that they are green when the normal crop is harvested. The grain from such green plants shrivels badly, and much of it is lost in thrashing. When much of a field is affected by the disease and there has been considerable recovery, the greatest quantity of grain is saved by delaying harvest until the diseased areas are ripe. It frequently is more economical to take a small loss, due to the shattering of overripe healthy plants, than to harvest the recovered plants while green. In some cases, where diseased areas in a field are more or less segregated, it is possible to harvest the healthy and diseased areas separately, thus reducing losses to the minimum.

## HOST PLANTS

Investigations carried on thus far have not shown any other crop than wheat to be positively affected by the rosette disease. Results of attempts to infect various plants will be found in Table II. There is some suggestion that rye may be infected, but this is not certain. While further investigations are being conducted to throw additional light on the host range of the disease, it is considered unnecessary to include plants outside the grass family until more is known about the cause of the disease.

TABLE II.—Percentage of rosette disease in various crops planted on infested land, Granite City, Ill., in 1919

Crops.	Percentage of rosette disease.	Crops.	Percentage of rosette disease.
Winter wheats listed in Tables VII and VIII (1920-21).....	0 to 98	Buckwheat.....	0
Marquis spring wheat.....	?	Flax.....	0
Durum spring wheat.....	?	German millet.....	0
Illinois No. 1 spring wheat.....	?	Hungarian millet.....	0
Red Fife spring wheat.....	?	Barnyard millet (Billion Dol- lar grass).....	0
Spring emmer.....	0	Timothy.....	0
Winter emmer.....	0	Awnless rye-grass.....	0
Spelt.....	0	Italian rye-grass.....	0
Hanna barley.....	?	English rye-grass.....	0
Hannchen barley.....	?	Bermuda grass.....	0
Svanhals barley.....	?	Crested dog's-tail.....	0
Wisconsin Pedigree barley.....	?	Meadow foxtail.....	0
Rosen rye.....	0	Meadow fescue.....	0
Common rye.....	0	Red fescue.....	0
Spring rye.....	0	Red top.....	0
Texas Red oats.....	0	Tall meadow oat-grass.....	0
Big Four oats.....	0	Kentucky bluegrass.....	0
Iowa 105 oats.....	0	Spring vetch.....	0
Sixty Day oats.....	0	Alfalfa.....	0
Iowa 103 oats.....	0	Sweet clover.....	0
Corn (maize).....	0	White clover.....	0
Kafir.....	0	Cowpea.....	0
Feterita.....	0	Navy bean.....	0
Amber sorgo.....	0	Rape.....	0
Milo.....	0	Sunflower.....	0
Sudan grass.....	0	Giant beggarweed.....	0

Certain varieties of spring wheat and of barley develop symptoms resembling the rosette disease of winter wheat, but there are certain differences which make it questionable whether these crops are affected by the disease.

#### DESCRIPTION OF THE DISEASE

During the first year's investigations it was not possible to make a complete study of the symptomatology of the disease, owing to the fact that the work was not started until May, fully seven weeks after the disease made its appearance in the spring. At that time field spotting was rather evident, and diseased plants showed a characteristic stunted development and the production of an excessively large number of secondary tillers, giving the plants a rosette appearance. These were the field and plant symptoms which attracted attention to the disease and which made it conspicuously different from other wheat diseases heretofore definitely known in this country. A rotting of the underground portions of tillers had started, but this condition was suspected to be a secondary effect rather than a primary cause, as observations, made earlier in the season (April 21) by A. G. Johnson, had not revealed a distinct rotting of the tissues in all cases, even though conspicuous signs of the disease, dwarfing and excessive tillering, were noted by him.

In 1920 the field studies were begun in the latter part of March, just at the time wheat plants were beginning to show signs of spring development. These studies were conducted in experimental plots located near

Granite City, Ill. In 1920 the first signs of the disease became manifest on March 24, and by April 1 there was no question as to its presence.

During 1921 observations were made on the experimental plots located near Granite City, Ill., and also on plots maintained by the Indiana Agricultural Experiment Station near Valparaiso, Ind. In 1921 the disease made its appearance at Granite City during the latter part of February. This early development doubtless was due to the fact that the winter had been unusually mild. The disease has not yet been detected with certainty in the fall.

#### FIELD SYMPTOMS

Shortly after spring growth begins diseased areas present a very striking contrast with healthy parts of the field. Such areas show up as rather definite patches which vary in size and shape (Pl. 2). In some cases one diseased plant (Pl. 3, A) may be found among plants showing no signs of the disease. Frequently patches are found which are made up of only a dozen or so diseased plants, but usually the patches are considerably larger than this, and most commonly range from 3 feet to 20 feet or more in diameter. These patches sometimes are circular, but more commonly are irregular in outline. Where the disease is most destructive, more than 95 per cent of the area of the field may be infested. The marginal limits of diseased areas tend to be rather sharply defined in contrast with the rather undefined patching usually found in connection with poor soil conditions. The spotting caused by the disease is independent of topographical variations, and frequently patches are found to cover both poorly drained dead furrows and adjacent well-drained land. In the diseased patches, plants which partly recover tend to ripen later than healthy ones, causing a striking green spotting in the fields at ripening time. During wet seasons weeds become abundant in the disease-infested spots.

#### PLANT SYMPTOMS

The first positive indication of the disease, as the latter is now interpreted, consists in a retarding of the early spring development of the fall tillers. At this time no external lesions are consistently associated with the living tissues of the diseased plants (Pl. 1, B). Occasionally *Helminthosporium* lesions have been found on the subcrown internode.<sup>9</sup> As pointed out later, such lesions sometimes are discernible in the early spring on the dead sheaths of autumn leaves.

In the course of about a week after the first appearance of the rosette disease, affected plants tend to develop a dark, rather bluish green color, and numerous secondary tiller buds and tillers develop. The development of these secondary buds and tillers takes place around the crown of the plant, in a manner somewhat analogous to the twig proliferations occurring in peach rosette as described by Smith (37). The excessive number of tillers developed by diseased plants give them a characteristic rosette appearance. Table III gives results of tiller counts made on the healthy plants and plants affected by the disease.

<sup>9</sup> The term subcrown internode as used in this paper refers to the structure in the wheat seedling between the crown and the base of the coleoptile. Under ordinary conditions this internode<sup>1</sup> structure usually elongates to a greater or less extent.

TABLE III.—Comparison of tillering in healthy wheat plants and those affected by the rosette disease of wheat

Variety.	Locality.	Date of observations.	Total number of plants counted.	Average number tillers per plant.	
				Diseased.	Healthy.
Harvest Queen (Salzer's Prize-taker) <sup>1</sup> .	Madison Co., Ill.	Apr. 7, 1920	48	10.0	4.5
Harvest Queen (white-chaffed Red Cross). <sup>1</sup>	Porter Co., Ind.	May 7, 1921	54	9.2	3.3

<sup>1</sup> Local names: The white-chaffed Red Cross and the so-called Salzer's Prize-taker are considered to be the same variety grown locally under the different names. The preferred varietal name for these is Harvest Queen. The name "Salzer's Prize-taker" as used in this paper refers to the variety with white chaff and red kernels grown under the this name in southern Illinois. The original or true Salzer's Prize-taker is a variety with red chaff and white kernels. (See p. 794.)

At the time these spring tiller proliferations become pronounced the basal tissues of the fall tillers begin to take on a dull white to straw color, and in the course of another week or 10 days this tissue may start to turn brown and the whole base of the plant may become rotted (Pl. 1, C). This last condition seems to vary considerably in different localities under different conditions. In the vicinity of Granite City, Ill., this rotting is apparently much more prevalent than it is near Valparaiso, Ind.

Microscopic examinations<sup>10</sup> of the innermost tissues at the bases of stunted tillers of plants showing the early symptoms of the rosette disease have revealed the presence of yellowish brown necrotic areas located within the parenchymatous region. These necrotic areas seem to be similar to those found in the stems of corn plants and in sugar-cane plants affected by mosaic, as reported by Kunkel (15) and Matz (24), respectively. Examinations of the host cells in and adjacent to the necrotic areas frequently show intracellular bodies very similar to those found in tobacco, sugar cane, and corn plants affected by mosaic, as described by Iwanowski (11), Matz (24), and Kunkel (15). These bodies are also very similar to those described and figured by Lyon (17) in connection with the Fiji disease of sugar cane.

Root systems of plants in the early stages of the disease do not show external signs of infection or other injury consistently. In some cases lesions have been found on roots, but fully as many have been found on the roots of plants not showing the symptoms of the rosette disease. As the disease progresses the root systems of affected plants show a retarded development and become infected to some extent by various organisms.

In dry situations diseased plants do not tend to send up secondary spring tillers, and such plants usually die early, forming a drooping tuft of brown, dead leaves and tillers (Pl. 3, B). During periods of heavy rain in the spring, diseased plants may be washed and beaten out of the soil, leaving the ground bare between healthy plants. In situations of favorable moisture and high fertility diseased plants often recover. While the fall tillers of such plants usually are killed, the secondary tillers may develop into short culms (Pl. 4, B) and produce heads. Such heads usually are small and imperfectly filled (Pl. 4, A).

<sup>10</sup> Much of the microscopic work done in connection with this phase was carried on by Dr. Sophia Eckerson and Dr. R. W. Webb. A full account of this work will be published in a future paper.

Stevens (33, p. 259) recognized the development of extra tillers or "shoots" as a characteristic of the rosette disease (called footrot by him), but apparently he did not regard this as a primary characteristic. He considered the browning and rotting of the basal portions of the plants as the most "constant" character of the disease.

It is quite true that when the rosette disease is not observed until a considerable length of time after its first appearance in the spring, the darkening and rotting of the basal tissue might be considered, under certain conditions, as constant characters, but even these characters do not seem to hold in all localities where the disease occurs.

When the rosette disease, as it occurs in different localities, is taken into consideration, the most constant or characteristic symptoms which have been found in three years of close observation, consist of (1) the arrested spring developments, (2) the excessive tillering, producing a rosette appearance, and (3) the dark blue-green color of the foliage in combination with the characters mentioned above.

On the basis of our present knowledge, the presence of *Helminthosporium* lesions on, or a rotting of, the basal parts of wheat plants can not by themselves be considered diagnostic characteristics of the rosette disease. This *Helminthosporium* is found to cause considerable injury on many wheat varieties growing in the regions where the rosette disease occurs and which are not known to show the characteristic symptoms of rosette. Furthermore, this organism has been found attacking wheat in sections of the United States where the rosette disease is unknown.

It is not the purpose of this paper to deal with the take-all and footrot type of diseases occurring in the United States, but it does seem advisable to give the principal characteristics of these troubles as found in this country, in order that they may be distinguished from the rosette disease.

The field spotting caused by take-all and footrot is practically the same as that caused by the rosette disease. Field spots, however, tend to show up earlier in the spring in the rosette disease than in take-all and footrot. The first indication of the latter seems to be a yellowing of the affected plants, a condition not found in the rosette disease. Such yellow plants rapidly bleach out or develop a bronze color and die, after which they tend to remain erect in a rather stiff, upright position, or they may break over at the base due to a dry crownrot. When death is caused by the rosette disease, the plants turn brown without passing through the yellowing stage, and such plants droop to the ground and form a flat tuft of brown, dead leaves.

A black plate or scalelike mat of mycelium is commonly found at the base of the tillers of plants affected by the take-all and footrot type of maladies, but this condition has never been found associated with the rosette disease. Premature ripening or the development of "white heads" occurs in the former, but this does not occur in the rosette disease. Rather the opposite condition takes place—that is, delayed ripening of the partially recovered diseased plants.

In general it may be said that the rosette disease of wheat may be diagnosed with certainty in the spring before healthy plants reach the boot stage. After this period the disease may, under certain conditions, be indistinguishable from certain insect and other troubles. The comparative symptomatology of some of the insect maladies and the rosette disease will be taken up in a later paper prepared jointly between the



Bureaus of Plant Industry and Entomology, United States Department of Agriculture.

In certain particulars the early stages of the nematode disease of wheat resemble the early stages of the rosette disease. In each disease there may be retarded development of the plants in the spring and the leaves may develop a dark green coloration and become very broad and thick, but in nematode-infested plants there is, in addition, usually a marked crinkling of the leaves and stems, a condition not associated with the rosette disease. Also, the leaf and stem tissues of nematode-infested plants are very vegetative and succulent in nature, and they show a characteristic sparkling surface. None of these characters are associated with the rosette disease.

In reviewing the literature<sup>11</sup> it was found that certain diseases of sugar cane show marked resemblances in certain particulars to wheat rosette. The Fiji disease as described by Lyon (17) is especially similar, and likewise the Sereh disease described by the same writer has certain characteristics in common with the rosette disease.

In certain particulars the mosaic disease of cane and that of corn resemble the rosette disease even in the matter of leaf mottling. The latter character has always been observed by the writer in connection with wheat rosette, but since it also occurs in varieties not susceptible to rosette it is not known whether the condition is directly connected with the rosette disease. Evidence in hand suggests that there may be a connection.

#### CAUSE OF THE DISEASE

It should be noted that the development of typical plant symptoms of the rosette disease seems to be connected in some way with dormancy or low temperatures which occur during dormancy. When plants are not subjected to these conditions the characteristic plant symptoms do not ordinarily develop. Shortening the dormant period has given fair results, but until methods are perfected it will not be possible to make much progress by working under greenhouse conditions or by working in the open out of the regular season for the development of winter wheat.

While a number of theories have been advanced concerning the cause of the rosette disease, the exact cause is still undetermined. The disease has been variously attributed to winter injury and to abnormal soil conditions, such as depleted fertility, presence of toxic or injurious substances, unfavorable soil type, and improper drainage. Animal and plant parasites and viruses have also been considered the cause. Most of these possible causal agents have been tested either directly or indirectly, and certain conclusions have been reached. These agents are discussed under the general divisions (1) nonparasitic factors, (a) winter injury, (b) soil conditions other than parasitic; (2) parasitic factors, (a) animal, (b) plant; (3) viruses.

#### NONPARASITIC FACTORS

##### WINTER INJURY

During the season that the rosette disease was first reported (1919) it was generally considered, among many farmers, that the trouble was caused by the abnormally mild winter of 1918-19. While there is

<sup>11</sup> The writer is indebted to Dr. C. W. Edgerton for the use of his library in connection with these studies.

evidence that winter conditions do influence the development of the disease, there is no evidence that winter conditions are the prime cause. While the disease was first reported after an unusually mild winter, it has recurred each spring since that time. In this 3-year period, two mild winters and one severe winter have occurred, as shown in figure 1.

This figure shows the average monthly mean temperature curve for the 10 years previous to 1918 and the monthly mean temperature curves for the past 3 winter seasons in the vicinity of Granite City, Ill. These curves are based upon the daily temperature records published by the United States Weather Bureau at St. Louis, Mo.

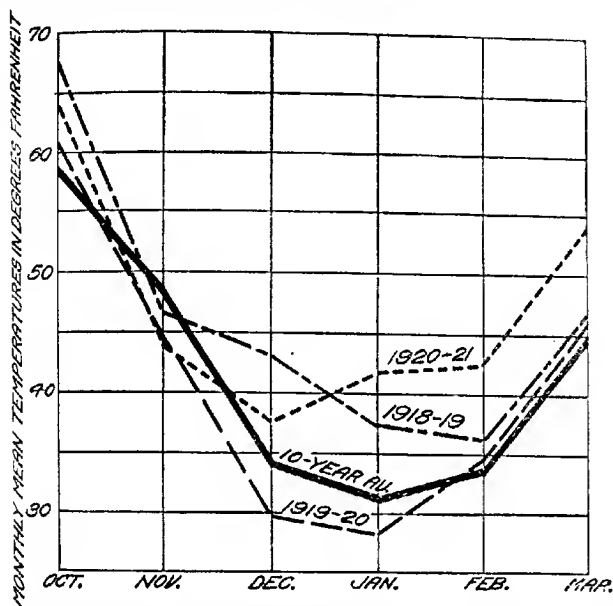


FIG. 1.—Graph showing monthly mean temperatures in degrees Fahrenheit for the six winter months in the years 1918-19, 1919-20, and 1920-21, compared with the 10-year average temperature in the same six months. The winters of 1918-19 and 1920-21 were unusually mild.

During the winters of 1918-1919 and 1920-1921 there was very little snow cover, and, as shown by the curves (fig. 1), these seasons were above normal in temperature. During the winter of 1919-1920, however, the opposite was true.

These data, together with the results from soil sterilization experiments discussed under Parasitic Factors on page 783, show that weather conditions in themselves are not the direct cause of the rosette disease.

#### SOIL CONDITIONS OTHER THAN PARASITIC

The rosette disease has been found to occur on all types of soil from a poor sand to a reasonably fertile gumbo, and under all conditions of soil drainage.

From the beginning of these investigations these factors were not considered very promising possibilities as primary causes of the disease;

first, because of the wide soil variations under which the disease occurred, and second, because characteristic symptoms of the diseased wheat plants are unlike the symptoms manifested by wheat plants suffering from a lack of fertility or from poor drainage. Plants suffering from the latter conditions usually make a poor start in the fall, and tillering is greatly reduced compared with that of plants growing in properly drained, fertile soil. Both of these symptoms differ from those found with the rosette disease.

While these observations practically eliminate soil type and drainage factors from the possible direct causes of the disease under discussion, they serve only as indirect evidence concerning the matter of soil fertility.

From the standpoint of nutrition and of soil toxins which may result from decomposition of organic or inorganic matter, or from plant root excretions, it was considered that if these factors were by any chance the primary cause of the rosette disease, the fact could be demonstrated by conducting simple fertilizer, cropping, and tillage experiments on infested land.

In light of the modern conception held by many workers in soil technology and allied branches, as Truog and Sykora (35), Lyon, Fippin, and Buckman (18 p. 136-138), Schreiner and Skinner (30), and Livingston (16), it was believed that some one of such a series of experiments would correct any soil disorders which might cause the disease and thus in turn materially control the disease.

In the fall of 1919, eight plots (30 by 44 feet) of uniformly infested land were used for the soil-treatment experiments.<sup>12</sup> Applications of fertilizers, lime, and manure were made on five of the plots, and the remaining three plots were left untreated for controls. All treatments were applied in the fall, either just before or just after seeding. Harvest Queen (Salzer's Prize-taker) wheat seed from a field free from the rosette disease was drilled across all of the treatments. Table IV gives the treatments, the rates of application of the materials, and the extent of disease which developed in the plots.

TABLE IV.—Data showing little influence of fertilizers, manure, and lime upon the control of rosette disease in Harvest Queen (Salzer's Prize-taker) wheat sown on uniformly infested land at Granite City, Ill.

Series.	Plot No.	Treatment.	Rate per acre.	Percentage of plants diseased.
1919-20	1	None.....		90
	2	Acid phosphate.....	222 pounds.....	92
	3	Rock phosphate.....	1 ton.....	97
		Stable manure.....	6 tons.....	
	4	Finely-ground lime stone.....	2 tons.....	93
		Rock phosphate.....	1 ton.....	
		Stable manure.....	6 tons.....	
	5	None.....		94
1920-21	6	Finely-ground lime stone.....	2 tons.....	96
	7	Stable manure.....	6 tons.....	93
	8	None.....		95
	1	None.....		85
	2	Potassium sulphate.....	500 pounds.....	87
	3	Sodium nitrate.....	440 pounds.....	84
	4	Acid phosphate.....	250 pounds.....	88
	5	Finely-ground lime stone.....	2.5 tons.....	

<sup>12</sup> All the field plot experiments referred to in this paper, unless otherwise stated, have been conducted on uniformly infested soil near Granite City, Ill.

Plainly the results from this series of experiments do not show any striking influence of these substances upon the development of the disease.

In the fall of 1920 this work was modified, using smaller plots and adding fertilizer materials in somewhat greater quantities per acre. The plots in this experiment were 4 by 6 feet in size, consisting of uniformly infested soil. Harvest Queen (white-chaffed Red Cross) seed from a field free from the rosette disease was sown in each plot on October 11. The fertilizers and lime, except the acid phosphate, were applied just before seeding, the latter was applied just after seeding.

Table IV gives the treatments, the rates of application, and the extent of disease which developed in the plots.

As in the previous experiment, the results from this series show practically no influence upon the development of the disease. The slight variations in the extent of disease developing in the different plots during both seasons is easily within the limits of experimental error. In fact, such small differences are not apparent when the plots are observed casually.

In order to determine any possible influence of cropping methods upon the disease, a plot of infested land was sown to various crops in the spring of 1919. Another portion of the infested land was summer fallowed and still another portion of it was left in winter wheat which was badly infested with rosette disease. Table V gives the arrangement of these plots and the crops used. In the fall of 1919, after plowing and fitting, these plots were sown crosswise with a 54-inch drill strip of Harvest Queen (Salzer's Prize-taker) wheat.

TABLE V.—*Influence of previous crops and summer fallow upon the control of rosette disease on infested land during the spring of 1920, at Granite City, Ill.*

Crop grown in spring of 1919.	Crop grown in fall and spring of 1919-20.	Percentage of wheat plants showing disease in 1920.
Potatoes.....	Harvest Queen (Salzer's Prize-taker) wheat.	98
Rape.....	do.....	98
Winter wheat (affected by rosette).....	do.....	94
Summer fallow.....	do.....	98
Buckwheat.....	do.....	97
Corn.....	do.....	93

In the spring the rosette disease developed with practically uniform severity on all the plots. Table V gives the relative percentage of the disease in each plot. While there was some variation in the percentage of disease occurring in these plots, the differences were very slight and the disease control was practically negative.

In the fall of 1920 Harvest Queen (white-chaffed Red Cross) seed was sown on infested land, part of which had been in summer fallow for two years and other parts in diseased and in healthy winter wheat the previous season. During the following spring the rosette disease developed in all the plots sown to the Harvest Queen (white-chaffed Red Cross) seed, and in no case was it possible to distinguish any difference in the percentage of disease present in the plots previously occupied by wheat and fallow. All contained from 90 to 95 per cent of diseased plants, showing conclusively that no control was obtained.

While many more fertilizers and combinations of treatments and more extended cropping measures could have been tested, it seems reasonable to believe that the experiments carried on covered a sufficiently large number of possibilities to warrant the drawing of rather definite conclusions therefrom.

While the extent of rosette disease was influenced very little in the various experiments just enumerated, the proportion of recovery after the development of the disease was somewhat less in the untreated than in the treated and fallowed plots.

In addition to the experiments on soil treatments and on rotation and tillage methods, soil disinfection experiments were carried on in connection with the work on parasitic factors. These experiments, which are described later, involved the treating of infested soils with a dilute formaldehyde solution previous to seeding in the fall. Such treatment resulted in a complete control of the disease.

While little investigation seems to have been made on the chemical reaction of formaldehyde in the soil, it appears to be rather generally believed that its action is confined largely to protein substances and that its chief effect in the soil is upon living organisms present.

The results of the experiments upon soil treatment and cropping methods and those obtained from soil disinfection, together with all the indirect evidence presented, seem to warrant the conclusion that improper nutrition or toxic substances, which may be in the soil as a result of continuous cropping or from the decomposition of organic or inorganic matter, are not to be looked upon as primary causes of the rosette disease.

#### PARASITIC FACTORS

This factor has been given more serious consideration than any other for the reason that a greater mass of indirect evidence points in its direction.

In order to throw some light upon this phase of the problem, it was decided to conduct experiments along lines of soil disinfection. It was reasonable to believe that if the disease is caused by certain types of parasites, soil disinfection should control the trouble. Two experiments were conducted on infested soil in the experimental plots located near Granite City, Ill.

The first experiment was conducted in six standard No. 00 galvanized steel garbage pails, 10½ inches in diameter by 11 inches deep. These pails were filled with heavily infested soil. The soil used in three of the pails was disinfected with a solution consisting of 1 part of 40 per cent formaldehyde and 49 parts water. This solution was sprinkled on thin layers of the infested soil as the soil was shoveled into the pails. Sufficient solution was used to produce a thick mud. This was done five weeks previous to sowing the seed. The three pails containing the untreated soil were handled in the same manner except that water alone was added to the soil. Pails were allowed to stand in the open to air and dry out for about five weeks. Harvest Queen (white-chaffed Red Cross) wheat, a susceptible variety, from a field free from rosette disease, was sown in each pail on October 8, 1920. The pails were set on a frame 1 foot from the ground and surrounded with sawdust held within a retaining wall (Pl. 5, A). This arrangement served to prevent unduly severe freezing and winterkilling. From 20 to 26 robust plants developed in each of the pails during the fall. No signs of the rosette disease developed during the fall period. In the spring, however, the disease devel-

oped in 92 per cent of the plants in the pails containing undisinfected soil. In the disinfected soil all plants were healthy and remained so during the season.<sup>13</sup> Plates 5, B, and 6 show the photographic evidence obtained from this experiment.

The second experiment consisted of two plots, A and B, each 4 by 6 feet in size, surrounded by wooden frames made of pine boards three-fourths of an inch in thickness and 8 inches wide. These frames were sunk in the soil 4 inches, leaving a 4-inch wall above the soil to keep out surface water (Pl. 5, A).

The soil inside these frames was heavily infested, like that used in the first experiment. In plot A, the dry surface soil was removed and a three-fourths-inch layer of the subsurface soil was removed and kept separate and not disinfected. The remaining soil was then removed from both frames to a depth of 10 inches, after which it was replaced in thin layers which were saturated with the formaldehyde solution previously described. Care was taken to return the soil layers in the same order as they were removed so that the original strata might be maintained as nearly as possible. After the disinfection the plots were allowed to air and dry for five weeks, after which the undisinfected layer of infested subsurface soil was returned to the surface and seed trenches of plot A. Then Harvest Queen (white-chaffed Red Cross) seed from a field free from the rosette disease was sown in rows 6 inches apart in both plots. Good germination was obtained and the resulting plants were very robust in the fall, showing no signs of rosette disease. In the spring essentially the same results were obtained as in the first experiment—that is, in plot A a high percentage of disease occurred, while plot B was essentially healthy. In the latter plot, however, a very few diseased plants developed around the edge, due doubtless either to splashing of water or seepage, or both, from the infested soil outside, indicating that the causal factor was disseminated to a slight extent in some such manner, apparently in the soil water. Apart from these few diseased plants, the plants in plot B were healthy and robust, remaining so throughout the season (Pl. 7, B). Plot A, which received the infested soil before seeding, developed the disease in about 75 per cent of the plants (Pl. 7, A).

Wheat growing in the infested soil just outside these plots developed from 85 to 95 per cent of rosette disease.

These results seem to prove conclusively that the rosette disease is caused by some factor other than winter conditions, soil type, or improper drainage; and, in view of the conclusions drawn in connection with soil factors other than parasitic, it seems that the evidence points most strongly in the direction of a causal organism or perhaps to some virus which may be greatly influenced by formaldehyde.

#### ANIMAL PARASITES

Insect parasites have been considered the cause of rosette disease by a number of investigators other than entomologists. A number of entomologists have examined plants affected by the rosette disease, and all have expressed the idea that insects are not the cause. In order to accumulate definite evidence on these points investigations have been conducted cooperatively by the writer and W. H. Larrimer of the Bureau of Entomology, United States Department of Agriculture. The results of these investigations will be published in a separate paper.

<sup>13</sup> Similar experiments carried on later with steam-sterilized soil gave the same results.

## PLANT PARASITES

Close observations have been made for evidences of bacteria and fungi. While the first group has not yet been found consistently associated with the disease at any stage of its development, certain fungi have been found under certain conditions associated with the diseased plants in the later stages of their development, and also with the plants in the fall previous to the development of the rosette disease in the spring.

All of these parasitic forms, however, have been found associated to a greater or less extent with plants not showing rosette disease. Owing to the fact that the disease was not reported until rather late in the spring of 1919, investigations were not started until May of that year. Previous to starting the investigation and also afterwards, the pycnidia of *Septoria tritici* Desm. were found to be rather numerous on the leaves of many plants affected by rosette disease and also on the leaves of plants not showing the disease in question. This organism, however, was not consistently associated with all plants showing the rosette disease. In the spring of 1920 this organism was not often found in the field, probably on account of unfavorable winter conditions. During 1921 it was again prevalent in the experimental plots and elsewhere. Although this organism seems to be a leaf parasite primarily, the question of its possible parasitic relation to the roots and crown of the wheat plant is being investigated.

Upon starting the work in May, 1919,<sup>11</sup> it was found that a *Helminthosporium* of the *Helminthosporium sativum* type was almost always present within the tissues of diseased plants collected near Granite City, Ill., and the same organism was found associated to some extent with plants affected with the rosette disease from other parts of Illinois and from Indiana. On the other hand, the organism also was found rather prevalent upon plants free from the rosette disease. Many other forms of fungi were isolated from the basal tissues of plants showing the advanced stages of the rosette disease, but these were in the minority and less consistently associated with the diseased plants.

At that time of year (May) the presence of *Helminthosporium* in the tissues of plants affected by rosette disease was not considered especially significant, as this organism was not confined to plants showing the disease in question, or to the infested fields. Further, this organism had been known for a considerable time to be pathogenic on wheat, according to Johnson (13) and Bolley (1) and to be responsible for many of the wheat troubles in the winter- and spring-wheat belts, but these troubles differ in a number of respects from the rosette disease.

During the season of 1920 an intensive field study of the disease was made by the writer. Observations were started on March 24, just as wheat was coming out of the dormant condition. As soon as the disease made its appearance (March 26) plantings were made from the basal tiller and root tissue of both diseased and healthy plants. This planting was conducted by the writer in the field laboratory located at Granite City, Ill., and by Mr. R. W. Goss, then with the Office of Cereal Investigations, located in the Department of Plant Pathology at the University of Wisconsin. Material used in the field laboratory was plated out within from one to two hours after the plants were taken from the soil. Material sent to the Wisconsin laboratories was collected just in time to make the

<sup>11</sup> Credit is due Mrs. Edith Seymour Jones for assistance in connection with the laboratory work done during the spring and summer of 1919.

best train connections and sent by special mail delivery. Ordinarily such material was in transit about 36 hours. The results of these platings have shown no organism to be consistently associated with the vital crown tissues of plants in the early stages of the development of the disease. By April 15 the same *Helminthosporium* which was found during the previous season made its appearance in the vital tissues of diseased plants and also on the tiller bases and the leaves of plants free from rosette disease. From this time on through the season this organism was found to be rather consistently associated with the disease. This was the period corresponding to the time when the work was started the previous year.

During the spring of 1921 a large number of platings were made from plants in all stages of the disease. Material was obtained from the experimental plots located near Granite City, Ill., and from the Indiana Agricultural Experiment Station plots located near Valparaiso, Ind. The plating was done by Dr. R. W. Webb in the laboratory of the Missouri Botanical Garden, at St. Louis, Mo., and by the writer in the laboratory of Plant Pathology at the University of Wisconsin. The results of these platings, which involved various media, reactions, and growing temperatures, were the same for the Granite City material as those obtained the previous year. In general, it may be stated that *Helminthosporium* was much less consistently associated with the diseased plants collected in Indiana than with plants collected near Granite City, Ill. The results of early platings were the same for material from both sources.

Experiments on the pathogenicity of pure cultures have been carried on with the predominating organisms isolated from diseased wheat plants. Of these organisms the *Helminthosporium* has been given the greatest attention. The results of this work show that this *Helminthosporium* is pathogenic on wheat, causing injuries which are apparently identical with those which heretofore have been produced by Johnson (13) with a similar or identical *Helminthosporium* isolated from wheat plants.

The types of injury produced by the *Helminthosporium* isolated from wheat plants in the late stages of rosette disease may be summarized as follows:

- (1) Mild to severe infection of the coleoptile and first leaf of the germinating seed, often causing death of the seedling before emergence.
- (2) Mild to severe root injury.
- (3) Mild to severe infections of the underground leaf sheaths and tillers, from the seedling stage through the preboot and heading stage.
- (4) Infection of leaves, causing characteristic lesions which may occur anywhere at the ligule or on the blade.
- (5) Infection of leaf sheaths above ground, causing lesions very similar to those produced upon leaves; also infection of the nodes and internodes.

The same types of injury have been produced by the same type of *Helminthosporium* isolated from Wisconsin wheat not showing the symptoms of the rosette disease of wheat. Similar results also have been obtained by Stakman (32) with a Minnesota strain of *Helminthosporium* which appears to be very similar, if not identical, with the strains used by the writer.

While Stevens (34) claims to have proved that the rosette disease (called footrot by him) (33) is caused by *Helminthosporium*, it is not clear that his evidence warrants this conclusion. Careful examination made by the writer of Dr. Stevens' diseased plants from inoculation experiments<sup>15</sup> revealed only seedling and young-plant injuries identical with



those referred to above in connection with the writer's inoculations with *Helminthosporium*. It is entirely possible that Dr. Stevens restricts the name footrot to these limited types of plant injury, but if so the fact does not seem to have been made clear in his papers. Further, both in personal conferences and in public meetings subsequent to the publication of his second paper (34), Dr. Stevens has stated that he has obtained from inoculations with *Helminthosporium* only the seedling and leaf lesions and that he has not obtained from pure-culture inoculations the dwarfing and excessive tillering, the diagnostic characteristics of the rosette disease.

While experiments on pathogenicity conducted with the various organisms isolated from plants affected by the rosette disease have thus far failed to reproduce the characteristic symptoms of the disease, the writer does not feel justified in assuming that none of the organisms tested are the direct cause. As pointed out earlier in this paper, experience with the winter-wheat plant shows that the peculiar symptoms manifested by this disease do not develop readily under greenhouse

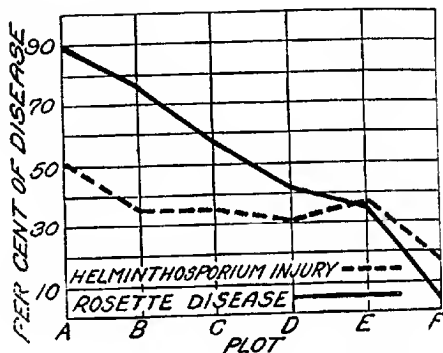


FIG. 2.—Graph showing tendency toward a correlation between the extent of rosette disease and the extent of *Helminthosporium* infection on wheat plants not showing the rosette disease in six experimental plots at Granite City, Ill.

conditions, and consequently further pathogenicity experiments are necessary before definite conclusions can be drawn.

A tendency toward a correlation between the percentage of *Helminthosporium* and of the rosette disease is shown in figure 2. The curves in this graph represent the percentage of both manifestations upon wheat plants growing in six date-of-seeding plots, the infestation of rosette disease in the soil being greatest in series A and rather gradually decreasing toward series F. The percentage of *Helminthosporium* injury in the various series was based on plants which escaped the rosette disease, thus enabling a direct comparison of results. On the whole the correlation between the two manifestations is rather striking and suggests a possible connection between *Helminthosporium* and rosette disease.

In following the development of *Helminthosporium* infection on wheat growing in the plots near Granite City, Ill., and elsewhere, it is found that infection takes place in the fall. Usually this infection obtains in the

<sup>12</sup> Exhibited at the Chicago meetings of the American Phytopathological Society in December, 1930.

coleoptile tissues and in the outer underground leaf sheaths of the tillers. Sometimes a mild to severe infection takes place in the subcrown internode, but apparently this is not as common as the infection of the other tissues mentioned. Fall infection seems to be rarely severe, and in most cases tiller lesions do not penetrate beyond the first or second leaf sheath. Winter wheat sown early shows a higher percentage of *Helminthosporium* infection and also more severe injury therefrom than does late-sown wheat.

A close study of the healthy wheat plant late in the fall and through the winter indicates that practically all of the outer leaf sheaths disintegrate and become separated from the vital tissues of the tillers or in some cases disappear entirely. Similarly, in plants showing *Helminthosporium* infection on the outer leaf sheaths in the fall, these outer leaf sheaths disintegrate, leaving the inner living leaf sheaths practically free from lesions. This explains the comparative absence of *Helminthosporium* on and in the vital tissues of wheat plants early in the spring. In this connection soil-temperature experiments conducted under controlled conditions show that the minimum temperature for the development of the wheat plant is somewhat below that for the development of *Helminthosporium* infection. This relationship probably explains why *Helminthosporium* does not begin its spring attack on the vital tissues of the plant until some little time after the wheat plant begins its spring development and also the increased fall infection of *Helminthosporium* in early sowings.

A condition somewhat similar to rosette disease has been noted by the writer on Marquis spring wheat growing in soil badly infested with *Helminthosporium*. Stakman (32) also reports a similar condition in spring wheat growing in *Helminthosporium*-infested soil in Minnesota. While this condition does resemble rosette disease in certain respects, it differs in others, as pointed out by Stakman.

Preliminary field studies made during the early winter of 1920-21 on Harvest Queen (white-chaffed Red Cross) wheat plants growing in soil known to produce the rosette disease showed that plants with fall infection of *Helminthosporium* on the subterranean portions of the tillers and on the subcrown internodes produce more tillers than plants free from such infection. Actual counts showed 42.5 per cent more tillers on the infected plants than on healthy ones.

In order to determine the influence of *Helminthosporium* upon tiller development, sterilized soil was inoculated in the greenhouse with a heavy water suspension of the conidia of the *Helminthosporium* isolated from a plant affected by rosette disease. Another plot identical with the former in every way except that the soil was not inoculated was used for the control. These plots contained about 10 square feet each. Harvest Queen (white-chaffed Red Cross) wheat (susceptible to rosette disease) and Marquis spring wheat (susceptibility questionable) were sown in both plots. The same number of seeds was used in each plot and for each variety, and seeding was done under the same conditions in each plot. The results of this experiment, given in Table VI, show a stimulation in tiller development in plants of the Harvest Queen (white-chaffed Red Cross) wheat infected with *Helminthosporium*, but not in Marquis.

While an increase in the number of tillers was obtained in Harvest Queen (white-chaffed Red Cross), this was noticed only by making

careful counts, as there was no striking indication of the characteristic stunting or blue-green color which occur on plants affected by rosette disease. Comparisons between Tables III and VI will show that while there is an increase in tiller development from the *Helminthosporium* inoculations, this increase is somewhat less than that found in plants showing rosette disease under field conditions.

While these are only preliminary data they indicate that *Helminthosporium* is capable of stimulating tiller development.<sup>16</sup>

TABLE VI.—*Influence of Helminthosporium infection upon tiller development in Marquis and Harvest Queen (white-chaffed Red Cross) wheat*

Variety.	Susceptibility to rosette disease.	Number of plants.		Reduction in stand on inoculated series.	Average number of tillers per plant.	
		Inoculated series.	Uninoculated series.		Inoculated series.	Uninoculated series.
Marquis.....	Questionable.....	240	263	Per ct. 8.7	2.66	2.03
Harvest Queen (white-chaffed Red Cross).	Positive.....	270	297	9.0	4.03	2.74

The correlations and other indirect evidences presented show that, under certain conditions at least, *Helminthosporium* is very closely associated with the rosette disease, and, as pointed out by the writer (20), there is a suggestion that the disease possibly may be an unusual manifestation of the *Helminthosporium* disease of wheat which has been known for some time to be present in several States (1), (10), (32), in the spring- and winter-wheat belts. Studies now under way will throw additional light upon the relation of this and other organisms to the development of the disease in question.

#### TOXINS AND VIRUSES

This group of factors has been considered among the possibilities of cause, and experiments now under way should throw some light upon it.

#### INTRACELLULAR BODIES

While it is possible that the intracellular bodies mentioned earlier in this paper may be some unusual type of organism which bears some relation to the cause of the rosette disease, it is also possible that they are reactionary bodies produced in the cells as a result of the disease.

Although these bodies have been found associated with the rosette disease, they have also been found associated with a mosaic-like leaf mottling in plants not showing the dwarfing characteristic of plants affected with the rosette disease. Thus far these intracellular bodies have not been found in wheat plants showing neither the rosette dwarfing nor mosaic-like leaf mottling. Further studies on both diseased and healthy plants are necessary before the nature of the bodies and their relation to the disorders can be definitely determined.

<sup>16</sup> Various workers, including Grantham (5) and Rimpau (28), have shown that tillering is stimulated by wide spacing between wheat plants. While the slight seedling killing in the inoculated plots resulted in a slight increased spacing, such spacing was practically the same in both varieties. Furthermore, no results have yet been found which indicate that such a small, irregularly distributed increase in spacing will increase tillering to the extent manifested in Harvest Queen (white-chaffed Red Cross) wheat.

### SPREAD OF THE DISEASE

#### BY INFESTED SOIL

This point has been proved by experiments which involved the transferring of infested soil to small plots of disease-free soil located in Alhambra, Ill., and at Madison, Wis., and by introducing infested soil into soil sterilized with a 1 to 49 solution of 40 per cent formaldehyde and water at Granite City, Ill. The latter experiment is discussed under the head of parasitic factors.

In all cases the typical symptoms of the rosette disease developed in the plots located at the two points given and in the soil-disinfection experiment where undisinfected, infested soil was introduced. In every case healthy plants developed in the local and disinfected soil.

The summer-fallow experiments discussed under soil factors other than parasitic show that the causal factor can persist in the soil for at least two years without apparent loss of its disease-producing powers.

#### BY INFESTED SEED

In 1920 seed was selected from plants which had practically recovered from the attack of the rosette disease. In the fall this seed was sown in field plots located at Madison, Wis., and Alhambra, Ill., and also in disinfected soil at Granite City, Ill. While the resulting plants were not robust, due to the poor quality of the seed used, all plots and soil containers were free from any indications of the rosette disease. Adjacent plots receiving the infested soil in each case developed the disease.

While these results demonstrate that the disease was not borne by the seed used in the experiment it is not felt that they are conclusive. Certain field observations lead to the belief that under certain conditions the disease is seed-borne. The foregoing results tend to indicate that the disease is not due primarily to a "running out" nor to constitutional weakness of the susceptible variety.

#### BY INFESTED STUBBLE

Parallel experiments with the stubble of diseased plants were carried on with the seed and soil transmission experiments, and, as in the seed experiments, negative results were obtained. These results are not considered conclusive, however, and this point is being investigated further.

#### CONTROL MEASURES

Various control measures have been under investigation, and distinct progress has been made in controlling the disease through the use of resistant or immune varieties.

As noted previously, various experiments on the applications of fertilizers, manure, and lime, and involving limited cropping methods, have failed to effect a control of the disease, although most of these have increased the percentage of recovery after the disease has developed. Seed treatments also have failed to effect a control. This is to be expected, however, as the disease is soil-borne. The burning of stubble and the application of iron sulphate to the soil failed to give control. Extremely late seeding controls the disease to a considerable extent. However, the seeding must be late enough so that the plants do not emerge until the following spring, and for this reason the method is not practicable.

## VARIETAL RESISTANCE

Extensive studies of varietal resistance have been made during the past two seasons. Many of the eastern wheat varieties have been grown on infested soil, and the disease has been controlled perfectly where resistant varieties have been used.

In 1919-20, 10 local varieties of wheat adapted to central Illinois were sown on uniformly infested land near Granite City, Illinois. The varieties were sown in adjacent parallel strips a drill-width (54 inches) wide and 50 rods long. The essential part of the writer's results from these experiments were first published by Reed and Dungan (27). Later the results were published by the writer<sup>17</sup> and by Humphrey, Johnson, and McKinney (10). These data are also given at this time in Table VII.

In 1920-21, 150 varieties and selections of winter wheat<sup>18</sup> were tested for susceptibility. Most of these were eastern varieties obtained from Dr. C. E. Leighty, of the Office of Cereal Investigations, but several were received from Mr. G. H. Dungan, of the Illinois Agricultural Experiment Station. All were sown by hand in rows 8 inches apart and 1 rod long, on uniformly infested land near Granite City, Ill. The varieties obtained from the Office of Cereal Investigations were sown in double rows and those received from the Illinois Agricultural Experiment Station were sown in single rows. A local susceptible variety, Harvest Queen (white-chaffed Red Cross), from a disease-free field was sown after every tenth variety throughout the series to serve as a control upon the uniformity of soil infestation. All control rows showed from 90 to 95 per cent of the rosette disease in the spring. Table VIII gives the list of varieties and selections and the percentage of disease developing in those which showed susceptibility. Owing to the limited size of these plots and to the fact that the stand in many of the plots was rather irregular, due to the very dry fall of 1920, yields were not taken on this series. Observations were made, however, on the general thrift of the varieties.

The outstanding feature of this experiment is the fact that only 6 per cent of all varieties showed susceptibility to the disease.

<sup>17</sup> McKINNEY, H. H. RESISTANCE IN WHEAT TO THE SO-CALLED TAKE-ALL DISEASE. In U. S. Dept. Agr. Bur. Plant Indus. Off. Cer. Inves. Cereal Courier, v. 12, no. 17, p. 229-231. 1920. Mimeographed.

<sup>18</sup> In a recent publication on classification of American wheat varieties (CLARK, J. ALLEN, MARTIN, JONES H., and BALL, CARLETON, R. CLASSIFICATION OF AMERICAN WHEAT VARIETIES. U. S. Dept. Agr. Bul. 1274, 238 p., 78 fig., 60 pl. 1921. Literature cited, p. 219-230), published since this paper was prepared, the following nomenclature has been proposed for certain of the varieties included in these experiments. In the following list the names used in this paper precede the corresponding ones used in the work cited: American Bronze=Prosperity; Budapest=Pesterboden; Crimean=Turkey; Dietz Longberry=Fulcaster; Early Harvest=Red May; Early May=Flint; Early Ripe=Red May; Enterprise=Red May; Everglades=Everglades; Fulcaster; Ghirka Winter=Alton; Harvest King=Poole; Hedge Prolific=Poole; Hungarian=Pesterboden; Indiana Swamp=Valley; Lancaster=Mediterranean; Malakol=Turkey; Michigan Amber=Red May; Michigan Wonder=Red May; Miller's Pride=Mediterranean; Minnesota Reliable=Turkey; Missouri Bluestem=Mediterranean; Niagara=Gipsy; Orange=Red May; Pearl Prolific=Currell; Pennsylvania Bluestem=China; Pride of Indiana=Red May; Red Cross=Red May; Reliable=Gipsy; Stoner (Marbleous)=Fulcaster; Stoner=Fulcaster; Super (Burbank's)=Jones File; Theiss=Turkey; Velvet Chaff (Pequette)=Pequette; Winter File=Jones File; Worlds Champion=Turkey.

TABLE VII.—Percentage of rosette disease infection, acre yield in bushels, and bushel weight of 10 varieties of winter wheat grown in plots 54 inches wide by 50 rods long, on uniformly infested soil, at Granite City, Ill., in 1920

Variety.	Infection by rosette disease.	Acre yield.	Bushel weight
		<i>Bushels.</i>	<i>Pounds.</i>
Harvest Queen ("Salzer's Prize-taker")	90 per cent	8.87	54.75
Red Wave	0	28.33	56.75
Illini Chief	25 to 30 per cent	24.08	58.00
Harvest King	Trace	28.83	56.50
Early May	0	25.61	58.50
Harvest Queen (white-chaffed Red Cross)	78 per cent	13.51	53.00
Dietz	2 per cent	26.20	59.50
Winter Fife	2 plants	32.87	58.50
Turkey	0	21.50	60.00
Calcester	2 per cent	25.80	60.75

TABLE VIII.—Percentage of rosette-disease infection in 150 varieties and strains of winter wheat grown in rod rows in uniformly infested soil near Granite City, Ill., in 1921

Variety.	Seed source.	Percentage of disease.
<b>Susceptible:</b>		
Harvest Queen (white-chaffed Red Cross)	Ill. Sta.	95
(Selection No. 13462, bearded, red chaffed)	C. I. 4834	95
Harvest Queen	C. I. 4882	75
Niagara (Sel. No. 13535)	C. I. 5307	70
Velvet Chaff (Penquite)	C. I. 3540	65
Missouri Bluestem	C. I. 1912	60
Dawson (Dawson's Golden Chaff)	Ill. 9-225	Trace.
	C. I. 6161	Trace.
Illini Chief <sup>1</sup>	Ill. Sta.	Trace.
Illini Chief	C. I. 5406	Trace.
Budapest	C. I. 5789	Trace.
Turkey (Wis. No. 18) <sup>1</sup>	Ill. Sta.	Trace.
World's Champion <sup>1</sup>	do.	Trace.
<b>Resistant:</b>		
Ahrens	C. I. 4848	0
Alabama	C. I. 5785	0
American Bronze	C. I. 5638	0
	Ill. Sta.	0
Beloglina <sup>1</sup>	C. I. 5964	0
Crimean	C. I. 5831	0
Crimean×Fultz (R. 5, 1919) <sup>1</sup>	C. I.	0
Crimean×Fultz (R. 6, 1919)	C. I.	0
Crimean×Fultz (R. 7, 1919) <sup>1</sup>	C. I.	0
Crimean×Fultz (R. 8, 1919)	C. I.	0
Crimean×Fultz (R. 9, 1919) <sup>1</sup>	C. I.	0
	C. I.	0
	C. I. 2906	0
Currell	C. I. 3326	0
	C. I. 4802	0
Currell×Fultz (R. 12, 1919) <sup>1</sup>	C. I.	0
	C. I.	0
Currell×Fultz (R. 13, 1919)	C. I.	0
	C. I.	0
Dietz Longberry	C. I. 1981	0
	C. I. 5387	0
Early Harvest	C. I. 4582	0
	C. I. 4582	0

<sup>1</sup>Especially robust.

TABLE VIII.—Percentage of rosette-disease infection in 150 varieties and strains of winter wheat grown in rod rows in uniformly infested soil near Granite City, Ill., in 1921—Continued

Variety.	Seed source.	Percentage of disease
Resistant—Continued.		
Early May.	Local.	0
Early Ripe.	C. I. 5319.	0
Enterprise.	C. I. 3399.	0
Evans.	C. I. 2946.	0
Eversole <sup>1</sup> .	C. I. 3011.	0
Fulcaster (R. 959, 1918).	C. I.	0
Fulcaster <sup>1</sup> .	C. I. 3013.	0
Fulcaster.	C. I. 3407.	0
	C. I. 4862.	0
	C. I. 1923.	0
Fultz.	C. I. 3349.	0
	C. I. 3423.	0
	C. I. 3594.	0
	C. I. 3598.	0
Fultz type (selection from C. I. 3604).	C. I.	0
Fultz type (R. 408, 1918).	C. I.	0
Fultz-Mediterranean.	C. I. 3421.	0
Ghirka Winter.	C. I. 1438.	0
	C. I. 3439.	0
Gipsy.	Ill. Sta.	0
	C. I. 3440.	0
	C. I. 5644.	0
Gladden.	Ill. Sta.	0
Gold Coin.	C. I. 5355.	0
Gold Coin (Junior No. 6).	C. I.	0
Golden Wave.	C. I. 6684.	0
Grandprize.	C. I. 5627.	0
Harvest Queen (white-chaffed Red Cross, Salzer's Prizetaker). <sup>1</sup>	Resistant selection.	0
Harvest King.	C. I. 5647.	0
Hedge Prolific.	C. I. 4859.	0
Hungarian <sup>1</sup> .	Ill. Sta.	0
Indiana Swamp (white-chaffed) <sup>1</sup> .	do.	0
Jones Fife <sup>1</sup> .	C. I. 5608.	0
Jones Fife.	C. I. 1942.	0
Jones Paris Prize.	C. I. 3568.	0
	C. I. 5146.	0
Kaured <sup>1</sup> .	Ill. Sta.	0
Kharkof <sup>1</sup> .	C. I. 5661.	0
Lancaster.	C. I. 1945.	0
Leap.	C. I. 5618.	0
Malakof.	C. I. 5663.	0
Malakof <sup>1</sup> .	Ill. Sta. No. 5-460.	0
Mammoth Red.	C. I. 2008.	0
	C. I. 3563.	0
Mealy.	C. I. 5404.	0
	C. I. 3565.	0
	C. I. 3407.	0
	C. I. 1395.	0
Mediterranean.	C. I. 3332.	0
	Ill. Sta.	0
Michigan Amber <sup>1</sup> .	C. I. 4864.	0
Michigan Wonder.	C. I. 3321.	0
Miller's Pride.	C. I. 4365.	0
Minnesota Reliable.	Ill. Sta.	0
New Amber Longberry.	C. I. 3561.	0
Nigger.	C. I. 5652.	0
Odessa.	C. I. 6151.	0

<sup>1</sup> Especially robust.

TABLE VIII.—Percentage of rosette-disease infection in 150 varieties and strains of winter wheat grown in rod rows in uniformly infested soil near Granite City, Ill., in 1921—Continued.

Variety.	Seed source.	Percentage of disease.
Resistant—Continued		
Ontario Wonder.....	C. I. 3843.....	0
Orange.....	C. I. 4868.....	0
Padui <sup>1</sup> .....	C. I. 6153.....	0
Palmer.....	C. I. 6685.....	0
Pearl Prolific.....	C. I. 3484.....	0
Pennsylvania Bluestem.....	C. I. 5342.....	0
Pesterboden <sup>1</sup> .....	Ill. Sta.....	0
Poole.....	C. I. 5653.....	0
Portage.....	C. I. 5370.....	0
Pride of Indiana.....	C. I. 3492.....	0
Red Cross (red-chaffed).....	(C. I. 5318.....	0
Red Hussar.....	(C. I. 3579.....	0
Red May.....	Ill. Sta.....	0
Red Rock.....	C. I. 5339.....	0
Red Russian.....	Ill. Sta.....	0
Red Wave <sup>1</sup> .....	C. I. 3497.....	0
Red Wave.....	C. I. 5624.....	0
Reliable.....	Ill. Sta.....	0
Rudy.....	C. I. 3508.....	0
(Selection) <sup>1</sup> .....	C. I. 5625.....	0
(Selection) (brown-bearded).....	C. I. 2908.....	0
(Selection).....	C. I. 3118.....	0
(Selection) (white-bearded).....	C. I. 3334.....	0
(Selection).....	C. I. 3135.....	0
(Selection).....	(C. I. 3068.....	0
(Selection) <sup>1</sup> .....	(C. I. 4131.....	0
(Selection).....	C. I. 3554.....	0
(Selection).....	C. I. 1593.....	0
(Selection from C. I. 3079).....	C. I.....	0
(Selection No. 131058).....	C. I. 6685.....	0
(Selection No. 13838).....	C. I.....	0
(Selection No. 131218).....	C. I.....	0
(Selection No. 131156).....	C. I.....	0
Stoner (Marvelous).....	(C. I. 3605.....	0
Stoner.....	C. I. 5961.....	0
Super (Rurbank's).....	C. I. 5961.....	0
Theiss.....	C. I. 2980.....	0
Treadwell.....	C. I. 5544.....	0
Triumph.....	C. I. 1561.....	0
Trumbull <sup>1</sup> .....	C. I. 3527.....	0
Tule.....	C. I. 3134.....	0
Turkey.....	C. I. 5657.....	0
Turkey <sup>1</sup> .....	C. I. 4140.....	0
Turkey.....	Ill. Sta. No. 514.....	0
Turkey <sup>1</sup> .....	Ill. Sta. No. 402.....	0
Turkey.....	Ill. Sta. No.....	0
Turkey.....	Ill. Sta. No. 10-110.....	0
Turkey.....	Ill. Sta. No. 12-41.....	0
Turkey.....	Ill. Sta. No. 509.....	0
Turkey (Wis. Ped. No. 2).....	Ill. Sta.....	0
Turkey (Iowa No. 404) <sup>1</sup> .....	Wis. Sta.....	0
Turkey <sup>1</sup> .....	C. I. 5580.....	0
Turkish Amber X Dale Gloria (R. 3, 1918).....	C. I. 6152.....	0
Turkish Amber X Dale Gloria (R. 4, 1919).....	C. I.....	0
Wheedling.....	C. I.....	0
Winter Chief.....	C. I. 4846.....	0
	C. I. 4878.....	0

<sup>1</sup> Especially robust.



A few of the varieties which showed susceptibility in 1920 did not show the disease in 1921. This occurred only among varieties showing low percentages of the disease in 1920. It is possible that the varieties showing the small percentage of infection were mixtures, while in those varieties showing high variations in the percentages of disease and non-susceptibility a genetic relation existed. This latter conclusion is based upon results obtained in selections studied in 1920-21 by the writer.

In all the experiments it has been found that certain plants within the Harvest Queen (Salzer's Prizetaker) variety (susceptible) always survived or escaped the rosette disease and that such plants showed the same general agronomic characters as the varietal type. At first it was thought that these were accidental escapes, but upon selecting the heads from such plants and sowing the seed from these on infested land (Pl. 8, A, a) 100 per cent resistance was obtained in all resulting plants and the head and kernel type remained true.

Further experiments are necessary to determine the resistance and susceptibility of additional varieties and to complete the study of many of the varieties and selections already tested.

It is evident from the results of the trials that the Harvest Queen (Salzer's Prizetaker and white-chaffed Red Cross), Illini Chief, Velvet Chaff, Missouri Bluestem, Niagara, and Indiana Swamp varieties are very susceptible to the disease and should not be grown in any of the districts where the rosette disease occurs.

The variety grown in central Illinois under the name Salzer's Prizetaker has white chaff and red kernels, and it is this variety which is susceptible. The original Salzer's Prizetaker was a variety with brown chaff and white kernels. This latter variety is identical with Gold Coin and Gold Coin (Junior No. 6), as listed in Table VIII, which have been found immune from the disease.

The susceptible variety Harvest Queen, known locally as Red Cross in central Illinois, also has white chaff and red kernels and is identical with the susceptible Salzer's Prizetaker variety. True Red Cross has red chaff and is a distinct variety grown to some extent in the eastern United States under the name of Red Cross. This variety was tested in 1921 and found to be resistant to rosette disease. All these Salzer's Prizetaker and Red Cross varieties are beardless. The variety known as Harvest Queen is supposed to be the same as the susceptible white-chaffed "Red Cross" variety. Harvest Queen is grown in the Missouri River Valley in eastern Kansas.

Certain of the resistant varieties are extensively grown in the infested area in Madison County, Ill., and the writer has made rather extensive observations on such varieties growing under farm conditions. Varieties which have been observed to maintain their resistance and show indications of immunity are Early May, Red Wave, and Jones Five. While all of these are good yielding varieties in central Illinois, it is doubtful if they come quite up to Harvest Queen (Salzer's Prizetaker and white-chaffed Red Cross) in all respects. The latter variety is a favorite among farmers who do not have the rosette disease on their farms. This variety stands the winter well, is very well adapted to the less fertile soils, yields well, and produces grain of very high quality. It is hoped that the resistant selections which have been made from Harvest Queen (white-chaffed Red Cross and Salzer's Prizetaker) will retain all of the good qualities found in the variety and that the resistance to the rosette disease will be found to be stable.

The outstanding result of the variety tests is that the great majority of winter wheat varieties are resistant to or immune from rosette. While this ratio between resistant or immune and susceptible varieties is unusual, it is paralleled in the cases of the Labaina, Sereh, and Fiji diseases of sugar cane.

#### INFLUENCE OF SEEDING DATE

In order that information might be obtained on this phase of the problem, a series of sowings was made during the fall of 1919 on land which developed uniform rosette infestation during the previous season. Two susceptible strains of Harvest Queen wheat (Salzer's Prizetaker and white-chaffed Red Cross) were used in the experiment. Each variety was sown on five different dates, except that, through error, Early May wheat was sown in the place of the first sowing of the white-chaffed Red Cross.

Each sowing consisted of a single strip 54 inches in width and 6 rods long. All seed was sown with an 8-spout disk drill. Seed was treated with formaldehyde and sown at the rate of 6 pecks per acre on September 18 and 24, October 4 and 16, and November 18. The results are given in Table IX.

TABLE IX.—Percentage of rosette disease developed in strains of Harvest Queen wheat (Salzer's Prizetaker and white-chaffed Red Cross) sown in plots 54 inches by 6 rods, on five different dates in 1919, at Granite City, Ill.

Strain of Harvest Queen wheat.	Percentage of disease in plots seeded on different dates.				
	Sept. 18.	Sept. 24.	Oct. 4.	Oct. 16.	Nov. 24.
Salzer's Prizetaker.....	95	90	90	79.0	4.5
White-chaffed Red Cross.....	No sowing.	78	78	73.8	0.28

In the first four sowings there was normal fall emergence, but from the sowing of November 18 emergence did not take place until spring. In the first sowings there was a little Hessian-fly injury in the fall. In the October 16 sowing considerable winterkilling took place. Both of these complications made it necessary to calculate the amount of Hessian-fly and frost injury in other neighboring plots not affected by the rosette disease in order that corrections might be made in the rosette disease plots.

Except in the November 18 sowing, a high percentage of disease developed in all the plots. Reference to Table X will give the exact percentage of the disease in each plot after corrections have been made for injury by Hessian fly and frost. It is quite evident that the date of seeding influences the development of the rosette disease, but this relation seems not to be of such nature as to permit economic application. It is remarkable that the disease should be so slight in the sowing that did not emerge until spring (Pl. 8, B).

The result of this field experiment shows very conclusively, on the basis of the November 18 sowing, that the problem is largely a seasonal one, and it bears out the general contention that the disease can not be produced successfully in a high percentage of plants under ordinary greenhouse conditions or in the field out of the regular season for the winter wheat plant.

Plate 8, B, shows the November 18 sowing as it appeared on May 27, 1920. While very little tillering took place on the plants in this plot, a very good quality of wheat was produced at the rate of 22 bushels per acre, with a bushel weight of 60.75 pounds. From the agronomic standpoint, this is remarkable for winter wheat sown on such a late date. For spring-sown winter wheat no such result is obtained. The resulting plants tiller vigorously, without shooting or heading, and produce no crop. The physiological basis for the behavior of the November 18 sowing affords a very interesting problem. Doubtless the effect of soil temperatures in relation to the stages of plant development is largely responsible.

The date-of-seeding experiment was repeated in the fall of 1920, using Harvest Queen (white-chaffed Red Cross) seed. The plots were the same width as those used the previous year, but they were only 2 rods long. Sowings were made on September 21, October 4 and 11, and November 4 and 19.

TABLE X.—*Influence of seeding date upon the percentage of rosette disease developed in Harvest Queen (white-chaffed Red Cross) wheat sown in plots 54 inches by 2 rods on five different dates in 1920*

Date of seeding.....	Sept. 21.	Oct. 4.	Oct. 11.	Nov. 4.	Nov. 19.
Percentage of disease.....	93.6	85.6	96.0	36.6	39.4

In connection with this date-of-seeding series it should be noted that owing to the mild winter the last two sowings made in November, 1920, emerged between Christmas and New Year's and a good stand resulted in the early spring.

The results from this series are shown in Table X. While a considerable percentage of rosette disease developed in the two latest sowings, it should be noted that the symptoms differed considerably from those manifested by plants in the early sown plots where normal fall emergence took place. The disease did not appear until fully six weeks after it had developed in the early sown plots. At this time the plants were from 12 to 16 inches in height. The first indications consisted in a retarding of certain plants followed by the dark blue-green coloration found in typical plants. Only slight excessive tillering resulted, and but few diseased plants developed normal heads. These same modifications in symptoms take place under conditions of reduced temperature in the greenhouse.

Just why a greater percentage of disease developed in the last two sowings made in 1920 than in the last sowing made the year previous can not be explained satisfactorily at this time unless it is for the reason that a steadier growth followed emergence in the sowing made on November 18, 1919, than with the last two sowings made in 1920. The latter sowings were subjected to a rather limited dormant period after emergence, thus simulating more nearly the conditions surrounding early fall-sown wheat.

It will be noted from Table X that there was an increase in the percentage of disease which developed in the sowing of October 11, 1920, compared with the two earlier sowings, thus causing a decided rise in the curve. It will be noted also from Table IX that there was a constant decrease in the percentage of disease in the 1919 plots from successively

later sowings. No exact explanation of this irregularity in the disease curves in the two seasons can be given, but it is noted that these curves follow closely the temperature curves for the germination periods for the several sowings. This relationship is very suggestive of a parasitic cause.

#### SUMMARY

This paper deals with the rosette disease of winter wheat, which was first reported in 1919 from Madison County, Ill., and a little later from Indiana under the name of take-all and later so-called take-all.

Investigations show that this disease is not the same as the take-all and footrot type of diseases which have occurred for many years in Australasia and Europe and have now been found in the United States.

The rosette disease has recurred in the field each year since it was reported, but the typical plant symptoms have not been reproduced satisfactorily under ordinary greenhouse conditions or in the field out of the regular season for winter wheat, thus reducing the problem to a seasonal one until such time as special methods have been perfected.

The origin of the rosette disease of wheat is unknown. It is believed that the disease has been present in certain of the infested areas for a considerable length of time.

So far as known this malady occurs only in the States of Illinois and Indiana.

Under conditions favorable for the disease it may be very destructive, causing a reduction of 75 per cent or more in yield in experimental plots. Under conditions favorable for the host, remarkable recovery from the disease may result and losses may be very slight. Forty per cent reduction in yield is not uncommon under farm conditions.

So far as definitely known the disease occurs only in certain varieties of winter wheat. Certain symptoms resembling the rosette disease have been observed in certain varieties of spring wheat and to a slight extent in barley and rye. No other grains or crops under experiment have developed symptoms resembling the disease.

The disease may cause a spotting in affected fields or it may be distributed rather generally over the fields. These spots or patches of diseased plants occur independent of any particular soil type or topographic condition in the field.

The most constant or characteristic plant symptoms consist of: (1) an arrested spring development, (2) an excessive tillering which results in a rosette appearance, and (3) a dark blue-green color of the foliage in combination with 1 and 2.

The take-all and footrot type of diseases cause field spotting, which is similar to the spotting caused by the rosette disease, except that the former diseases seem to appear a little later in the spring.

In the take-all and footrot type of diseases no excessive tillering has been noted, and diseased plants turn yellow shortly after the spring growth begins. Such plants gradually bleach and dry up, usually standing erect and assuming a stiff, wiry posture. Sometimes older plants break over at the base. On the other hand, plants affected by the rosette disease, when killed early, form a flat or drooping tuft of brown dead leaves which do not pass through a yellow stage.

A black "mycelial plate" is rather characteristic of the take-all and footrot type of malady, but this has never been found associated with the rosette disease.

Premature ripening or the development of "white heads" is commonly found in take-all and footrot, but in the rosette disease, delayed ripening occurs in those plants which partially recover from the disease.

The take-all and footrot type of trouble occurs in varieties of wheat known to resist the rosette disease.

None of the fungi commonly associated with take-all and similar footrots have been found associated with the rosette disease.

The rosette disease is diagnosed with greatest certainty in the spring before healthy plants reach the boot stage.

The rosette disease behaves in many ways similar to the Fiji, Sereh, and mosaic diseases of sugar cane and the mosaic disease of corn.

The cause of the disease is unknown.

Soil disinfection experiments made in the field show that a solution of 40 per cent formaldehyde and water (1 to 49) applied to infested soil will control the disease. The disease developed in nondisinfected plots of the same soil located directly adjacent to the disinfected soil. The same seed, Harvest Queen (white-chaffed Red Cross), was used in both cases.

A similar experiment conducted using steam-sterilized soil gave the same results.

The foregoing experiment together with winter temperature records proves that winter injury and temperature are not the prime causes for the rosette disease. This evidence, together with that obtained from fertilizer and fallowing experiments, indicates that nonparasitic soil factors probably are not the cause of the disease.

The results from soil disinfection point most forcibly to an organism or perhaps a virus as being the causal factor of the disease.

There is no indication that insects are the cause of the trouble. This view is maintained by a number of prominent entomologists.

Certain unusual intracellular bodies have been found associated with wheat plants showing the very early stages of rosette.

A number of fungi have been found associated with diseased plants during certain stages, but none has been found consistently associated with the trouble when it makes its first appearance in the spring.

It has been proved that the disease is soil-borne. While experiments have not shown that the disease is seed-borne, certain field observations indicate that it possibly may be.

The causal agent of the disease is known to persist in summer-fallowed soil for at least two years without apparent loss of its disease-producing powers.

The disease is controlled through the use of resistant varieties.

Only 6 per cent of the 150 varieties and selections used in experiments have shown definite susceptibility.

Extremely late fall seeding with spring emergence of seedlings practically controls the disease, but this method of control is not practicable.

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PLATE I

Healthy and diseased Harvest Queen wheat plants of same age.

A.—Healthy plant showing good development and normal green color.

B.—Plant showing early symptoms of rosette disease, namely, marked dwarfing, excessive tillering, blue-green color, and some leaf mottling before *Helminthosporium* invasion has started.

C.—Plant attacked by rosette disease, showing symptoms after *Helminthosporium* rot has developed. General symptoms similar to those in B, but with marked rotting and discoloration of basal portion.





PLATE 2

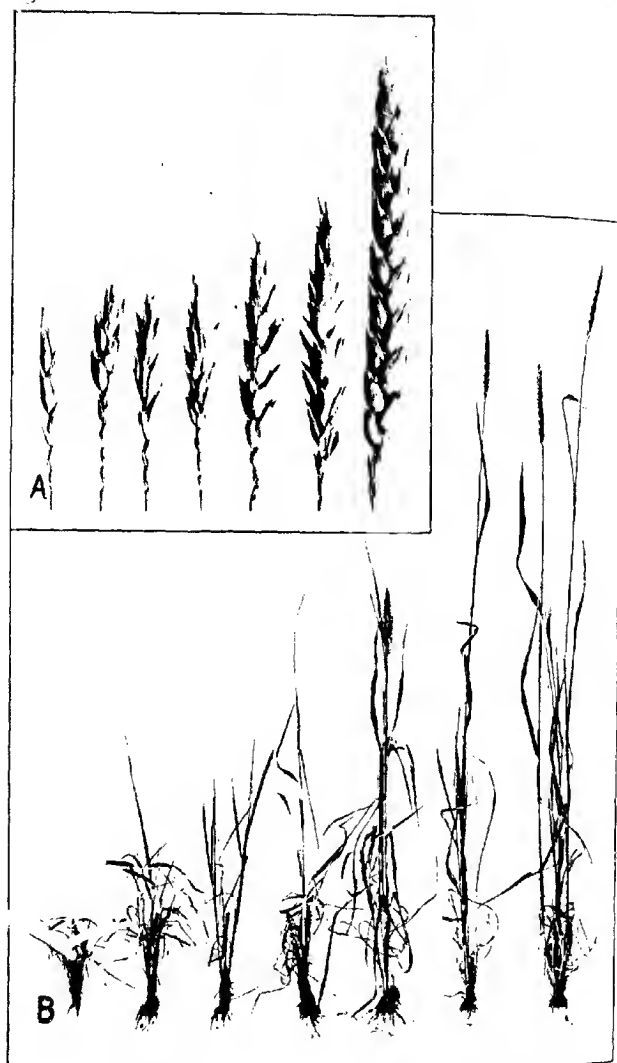
Spot in Field of Harvest Queen (Salzer's Prizetaker) Wheat Caused by the Rosette disease. Diseased plants (a) are greatly dwarfed in contrast with surrounding healthy ones.

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**PLATE 3**

Single wheat plant (A,a) attacked by the rosette disease among surrounding healthy ones, and bird's-eye view at harvest time of remains of wheat plants (B,a) killed by the rosette disease early in the season, with stubble of healthy plant at left.





#### PLATE 4

Heads and plants of Harvest Queen (Salzer's Prizetaker), diseased and healthy.

A.—Six poorly developed heads (left) from plants partially recovered from the rosette disease, and one typical healthy head at (right).

B.—Six plants (left), all of same age, showing various degrees of recovery from the rosette disease. Healthy plant at right.



PLATE 5

A.—General view showing boxed-in soil plots in foreground and at right and galvanized-iron pail series in background at left.

B.—At left, two pails containing healthy plants of Harvest Queen (Red Cross) wheat grown in infested soil that had been disinfected with formaldehyde previous to sowing the seed. At right, two pails containing plants of Harvest Queen wheat of same age grown in infested soil undisinfected. Healthy seed used throughout. Typical rosette disease developed only in the undisinfected soil.

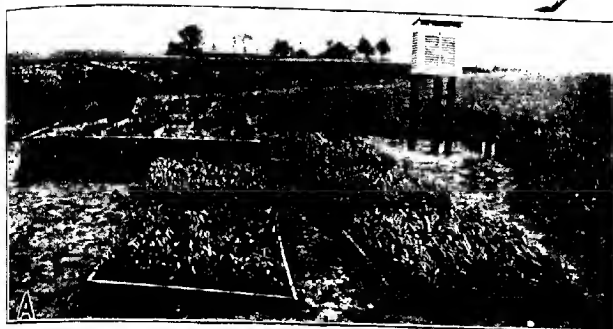




PLATE 6

Plants of Harvest Queen 'Red Cross' wheat from disinfected (left) and undisinfected (right) pails of soil.

A.—Healthy plants from one pail of disinfected infested soil shown in Plate 5, B at left.

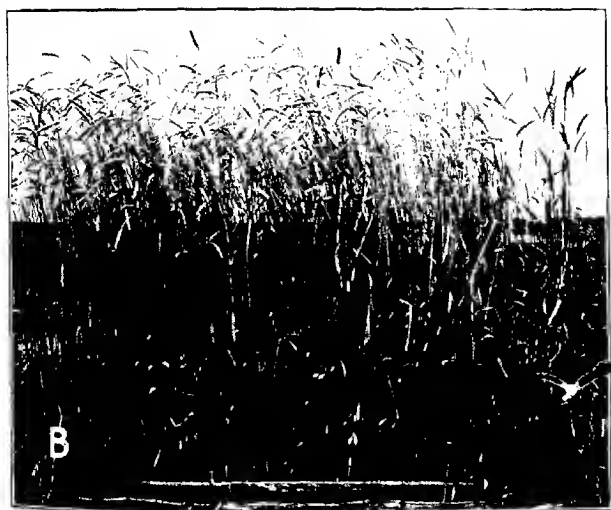
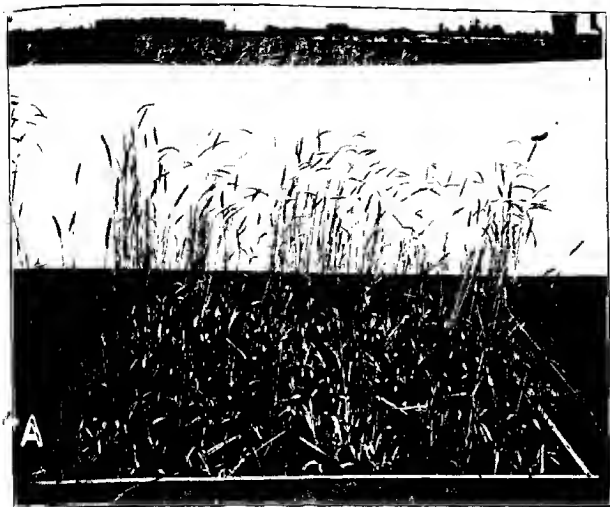
B.—Diseased plants from one pail of undisinfected infested soil shown in Plate 5, B, at right, showing severe attack of rosette disease.

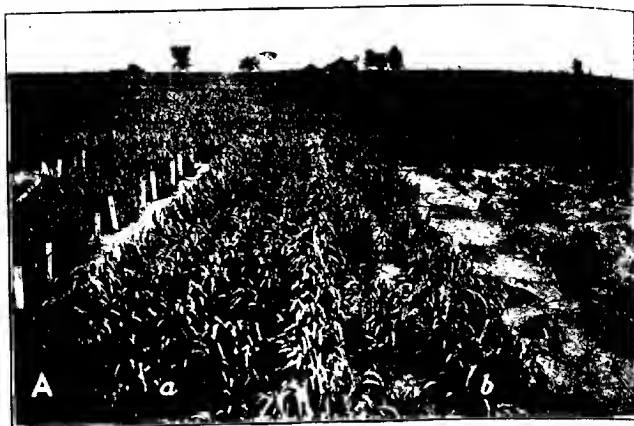
#### PLATE 7

Plants of Harvest Queen (Red Cross) wheat grown in plots of inoculated (A) and uninoculated (B) disinfected soil.

A.—Thin stand caused by severe attack of rosette disease. The soil in this plot had been disinfected in the same way as that in B, but was inoculated with a small amount of infested soil when seed was sown.

B.—Good stand of wheat. The soil in this plot had been disinfected with formaldehyde previous to seeding and was not inoculated. Both A and B were sown at same rate and at same time with clean seed.





#### PLATE 8

The control of the rosette disease by the use of resistant varieties and by very late fall planting.

A.—a, Healthy wheat plants grown from specially selected seed from resistant plants of Harvest Queen (Salzer's Prizetaker) grown on infested land; b, diseased wheat of same age and of same variety grown from unselected seed. Plot b showed 90 to 95 per cent of rosette disease.

B.—a, Good stand obtained on uniformly infested soil by very late fall sowing. This plot was sown November 18, 1919, and plants did not emerge until the following spring; b, thin stand of dwarfed wheat, heavily infected with the rosette disease. This plot was sown October 16, 1919, and the plants emerged in the fall. The thin stand is due in part to winter injury, but the dwarfing is caused by the rosette disease.





## ACCUMULATION OF ALUMINUM AND IRON COMPOUNDS IN CORN PLANTS AND ITS PROBABLE RELATION TO ROOTROTS<sup>1</sup>

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### INTRODUCTION

The rots of the roots and stalk parts of the corn plant present a very complex problem, the entities of which are difficult to isolate for study and interpretation. Certain fungi and bacteria, which are more or less common in all fields in which corn is grown commercially, are almost always present in these rotted tissues. At times the rot diseases may be very severe, but the damage caused by them seems to be influenced by certain soil conditions which are associated with deficiencies of the essential nutrients or with unbalanced combinations of available salts for absorption by the plants. Frequently the extent of damage may vary markedly in certain parts of fields or in different fields wherein the same seed stock had been planted.

One of the most common characteristics of root rotted plants and of plants growing in certain areas in some fields is the brown to brownish purple discoloration of the vascular plate tissues of the nodes, as shown in Plates 2 and 5. This nodal discoloration has been observed in plants in all stages of development. It has been found in plants growing in all parts of the Corn Belt.

The most common difference between plants which show indications of being diseased and those which are of normal growth and bear normally matured ears is this almost universal brownish purple discolored condition of the nodal tissues. Attempts to isolate organisms from these tissues frequently prove futile, although in a large majority of cases the common *Fusarium moniliforme* Shel., *Gibberella saubinetii* (Mont.) Sacc., a white bacterium, *Penicillium* sp., *Rhizopus* sp., and others are found to be present, especially if the nodal tissues in the lower parts of the stalk are cultured.

This nodal discoloration has been found in young plants which show no rot lesions whatever on the roots and from which no organisms could be isolated. Later in the season, however, abundant rootrots have developed in the plants, especially in "spots" in the fields where the majority of the plants showed these nodal discolorations, and much damage resulted. Field observations and experiments show that the largest numbers of seedling injuries also occur in the soils where these

<sup>1</sup> Accepted for publication July 23, 1927. The results reported in this paper have been obtained in investigations conducted jointly by the Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture, and Purdue University Agricultural Experiment Station.

nodal troubles are most abundant. The types of soil which are high in organic matter and low in calcium and phosphorus seem to be the best environment for the development of seedling-blight. Because of these relations an attempt was made to ascertain the cause of the brownish purple discolorations of the vascular plate. Microchemical tests made upon these discolored tissues proved the presence of organic compounds of iron and aluminum in them.

The purpose of this paper is to discuss the methods used to detect these metallic accumulations and the methods used to determine the cause of the nodal discolorations and subsequent disintegrations and their probable importance in relation to the development of the rootrots, stalkrots, and earrots of corn.

#### OCCURRENCE OF IRON AND ALUMINUM IN PLANTS

Iron compounds are essential to the normal development of all green plants. Their importance has been demonstrated by numerous experiments, the history of which needs no reference here. Chlorosis, which has been directly referable to conditions which develop in the plant and which is associated with deficient quantities of available iron salts, is well known and its causes are more or less understood.

Less is known regarding the occurrence of aluminum in plants, although considerable work has been done in the study of its rôle in the soil as a factor affecting the growth of plants, both as a stimulant and as a toxic agent.

Mazé (17)<sup>2</sup> believes that the presence of aluminum in the soil is necessary for the normal development of maize. He also includes iodine, fluorine, and boron as being necessary. True, Black, and Kelley (23) report a higher aluminum content of the tops of spinach when the plants are diseased than when they are healthy. Kratzmann (14) reports the ash content of the leaves of maize to contain 2.35 per cent of aluminum. A study of 130 different plants showed that aluminum is widely distributed, and the unusually large quantities present in some plants suggested that certain species may be "aluminumophilous." According to Kratzmann, different plants possess a specific elective power toward aluminum. Two plants of different species may accumulate widely different quantities of aluminum even though grown close together.

This same phenomenon of varying capacities for the absorption of aluminum by different plants seems to apply equally well to different individuals within a definite variety of corn, or even to individual plants grown from different kernels from the same seed ear. These differences may be interpreted as being due to the heterozygosity of the strain of corn. Qualitative tests for iron and aluminum applied to many plants studied in the rootrot investigations show very variable amounts of iron and aluminum in different stalks. From these observations it is evident that Kratzmann's designation of specific elective powers of plants for aluminum applies to corn for both aluminum and iron. The conditions which develop in the corn plant when large quantities of iron and aluminum salts enter the plant and concentrate in certain tissues have not been studied so far as can be learned.

<sup>2</sup> Reference is made by number (italic) to "Literature cited," pp. 822-823.

## SELECTIVE ABSORPTION IN RELATION TO RESISTANCE TO ROOTROTS

It is believed that resistance of the corn plants to rootrots may be closely related to what appears to be a selective absorptive capacity of plants toward aluminum and iron when these metals are available in subtoxic concentrations. Selective absorption may be a very important heritable character in corn. If so, the phenomenon of susceptibility to rootrots which is associated with the accumulation of abundant quantities of iron and aluminum in different parts of corn plants may have as one of its causes a definite type of selective absorption for aluminum and iron salts. Therefore, the accumulations will occur in the plants under conditions in the soil when the quantities of these available salts are in subtoxic proportions. Such conditions usually are found in most acid soils.

It is true also that the quantity of available aluminum compounds in some soils may be sufficient to more than overcome the natural resistance possessed by strains which ordinarily show good growth in soils with lower concentrations of available iron and aluminum salts. In this case, irrespective of strain differences, the plants may be severely affected and a distinct toxic effect of the aluminum be noted in all of them. Plate 4 shows two fields where many of the plants were severely stunted. All of these plants had the typical nodal discolorations and disintegrations of the basal tissues shown in Plate 2, B.

A field of Stowell's Evergreen Sweetcorn was seen at Decatur, Ill., in August, 1920, in which all the plants were stunted in that part of the field which had been in clover sod the preceding season. In the part of the field which had been in corn the plants showed good growth. The plants which were stunted showed concentrations of the metals in the nodal tissues, but no root lesions were noticed at that time. Later in the season many of these plants became severely rotted. The plants which had made better growth on the corn land were notably less seriously attacked. The soil was the typical Illinois black loam and gave an acidity reaction of  $P_H 5.6$  for the clover-sod land and  $P_H 6.2$  for the corn land.

The nodal-tissue injuries shown in Plate 2, B, and Plate 5 are not the only effects resulting from the accumulation of these metals in the corn-stalks. Analyses of the various plant parts show that the metals are distributed throughout the plant. The very obvious effects of the metals upon the nodal tissues gives them a seemingly greater importance in these tissues, but the unbalanced absorption of essential nutrients which takes place when these discolorations of the tissues are produced also accounts for a disturbance in the normal functioning of other active tissues.

Analyses of the leaves from diseased and healthy stalks show wide variations in the quantities of aluminum in them. The average aluminum ( $Al_2O_3$ ) content of the ash of the leaves of four selected normal stalks from a field near Battleground, Ind., in 1920, was 0.44 per cent, while the aluminum content of the ash of the diseased plant leaves was 0.98 per cent, over twice as much. The calcium ( $CaO$ ) content was 0.70 per cent in the normal leaves and 0.56 per cent in the leaves from diseased stalks.

The percentage of aluminum in the ash of the leaves from two root-rotted stalks from Bloomington, Ill., in 1918, was 1.16 per cent, while that of the leaves from stalks which remained healthy, and which were grown from the same seed ear, was 0.67 per cent. These cases which have just

been cited suggest that the influence of the aluminum salts may be harmful in the leaves and that, while the stalks which are diseased seem to have large quantities accumulated in the nodal tissues, these accumulations may indicate only the relative quantities which have been carried on to the leaf tissues to concentrate there and become toxic when in sufficient quantity. The problem of chlorophyll disturbances is a most complicated one, and the influence of these metals as catalysts upon the production of chlorophyll and as harmful agents upon the functioning of the chlorophyll tissues is little understood. The metals affect the chlorophyll tissues differently according to the amounts of alkaline bases present in the leaves.

#### AVAILABLE ALUMINUM SALTS IN SOILS

Much has been written of late relative to available aluminum and iron salts in the soil and their relation to soil acidity. Extracts from some acid soils have yielded quantities of aluminum salts in toxic concentrations. One of the explanations of the phenomenon of acidity in the soil is that it arises from the hydrolysis of salts of iron and aluminum.

In 1913, Abbott, Conner and Smalley (7) reported the results of their investigations upon an unproductive acid soil in northern Indiana. They found aluminum nitrate present in the soil extracts and attributed the acidity of the soil to the hydrolysis of the aluminum nitrate. Daikuhara (8) believes that salts of iron and aluminum are absorbed by the humic acids and other colloids of the soil and are set free again on treatment with neutral salt solutions. He infers that such compounds must exercise considerable influence not only on the acidity of purely mineral soils but also on that of soils containing humus. The injury to vegetation is thought to occur when the compounds of iron and aluminum absorbed by the soil colloids are liberated as soluble acid salts of iron and aluminum upon the application of mineral fertilizers.

In 1916, Conner (5) stated that much of the harmful acidity of acid soils is due to the presence of toxic acid salts of aluminum and iron and that the reduction of soil acidity by acid phosphate probably is due principally to a combination of the soluble phosphoric acid with the acid salts of aluminum and the consequent formation of insoluble nonacid compounds.

Spurway (22) attributes the acidity of certain glacial soils to the "hydrolytic-ratio" between the salts of the alkaline earth metals on the one hand and those of iron and aluminum on the other.

Hartwell and Pember (10) in 1918 have shown that the toxic effect of certain acid soils is due not to the true acids but to aluminum salts present in extracts from these acids soils. They checked the effects of aluminum salts added to nutrient solution with acid soil extracts and found the free acids were relatively harmless and that the toxicity was due to the active aluminum. This work was valuable because it made the plant test the final one for the effects of soil acidity.

Mirasol (18) has studied aluminum as a factor in soil acidity. He has given a very extensive bibliography of this subject and conducted experiments which showed the beneficial effects of applications of lime and acid phosphate in reducing toxicity due to aluminum compounds. These aluminum salts are claimed to be derived from the acid-soluble aluminum hydroxid, or gibbsite. In soils sufficiently provided with calcium, toxic aluminum salts may never be formed, but in soils deficient in calcium and other bases, such as acid soils, toxic aluminum salts are largely the end products of sulfofication and nitrification. Of

interest also is the fact developed by Conner (6) that the quantities of soluble iron in soils are greater when the soils are saturated.

Spurway (23) presents data relative to the influence of various salts on the release of soluble aluminum compounds in four soils which he studied. The general tendency of all of the treatments of these soils was to increase the quantities of soluble aluminum, the greatest effect being produced by calcium carbonate and acid phosphate. He reports also that hydrated lime increased the amount of aluminum in the first leaching, but a marked decrease occurred in the second leaching, 15 days after the treatment.

It is evident that the importance of available aluminum salts in the soil is beginning to be appreciated. Aluminum is found in practically all the soils in the Corn Belt, and it is certain, therefore, to become an increasingly important factor in soil management when a system is practiced which does not conserve the calcium and phosphorus salts.

#### DISTRIBUTION OF IRON AND ALUMINUM IN NORMAL AND DISEASED PLANTS

The tissues in which the metals, iron and aluminum, accumulate are the vascular plate tissues in the nodes of the stalk and shanks and the scutellum of the kernels, as shown in Plate 5. The metals also concentrate in the leaf tissues, as has been determined repeatedly by quantitative analyses. As a rule, the oldest leaves contain the largest quantities of these metals. Gile and Carrero (9) have shown that iron is immobile in the oldest rice leaves and in this respect is similar to silicon and magnesium.

The locations of the tissues in the stalk in which iron and aluminum accumulate are those referred to as "zone B" in Plate 3, A. These tissues appear white in normal stalks, with traces of yellow and sometimes green. When the metals concentrate in them they show various gradations of color and of disintegration. Plates 2, B; 5, A and B; and 6 show different kinds of injuries observed in plants in the field.

The extreme toxicity of copper sulphate is seen in Plate 3, B, where the tissues in both zone A and zone B have been seriously affected. This is the only metallic salt studied so far which affects both zone A and zone B tissues. The tissues of the basal nodes, shown in Plate 7, B, are the tissues in which the accumulations occur first. They are the oldest functioning nodal tissues, and their length of service, as indicated by a continued healthy appearance of the roots which have their origin in these tissues, seems to be related to the relative quantities of iron and aluminum which accumulate in them. Part of the basal tissues in the stalk shown in Plate 8, B, are not functioning, as shown by the dry appearance of the lowest and oldest roots. The plant was able, however, to produce additional roots and thereby continue vegetative growth.

In Plate 7, A and B, is shown the base of a stalk which manifested disease symptoms and which was barren. The basal tissues contained abundant accumulations of iron and aluminum compounds. Instead of the metals being distributed more or less uniformly throughout the stalk, as in the normal plant shown in Plate 9, the largest quantities were present in the lower nodal plates and these were brownish purple discolored, and disintegrated, as shown in Plate 7, C.

These two plants illustrate some of the differences commonly noted between plants which are resistant to the development of rots in the roots and stalks and those which become severely rotted. When the plants are vigorous and apparently healthy the quantities of the metals in the nodal tissues are nearly equal throughout the stalk and are not in sufficient quantity or in the state of combination with the plant tissues which causes the brownish purple discolorations.

Furthermore, the accumulation of the metals in the basal nodal tissues of stalks showing symptoms of disease does not necessarily mean a larger quantity of metals in the stalk as a whole. It indicates primarily an unequal distribution of the metals, which can be observed readily by using the microchemical tests, compared with the uniform distribution which is characteristic of normal plants.

The nodal plate from which the ear shoot originates frequently has the largest accumulation of all the nodes in the stalk. When this condition develops early in the life of the stalk the ears may form imperfectly. The nodal tissues may become disorganized and consequently weakened (Pl. 10, B), so that the shanks may break over before the ears are completely matured, as is shown in Plates 6 and 16, B. When this happens the shank scars will show the typical pinkish to brownish purple discolorations, the color varying in intensity according to the time when the discolorations begin to develop in relation to the degree of maturity of the ear.

This condition of the stalk is believed to be a very important factor affecting the rate of maturation of the ears. When the nodal tissues are affected, as shown in Plate 10, B, the ears are delayed in their maturity and tend to remain starchy. It has been found that the distribution of phosphorus also is affected in stalks with these accumulations of the metals. The phosphorus content of the nodal tissues is higher when the iron and aluminum compounds become concentrated in these tissues. The disorganization of the nodal plate tissues interferes with the movement of sufficient quantities of phosphorus and probably other materials to the developing ears and in this way may cause the ears on affected plants to remain starchy. The results of the investigations upon this interpretation of one of the causes of starchiness in corn will be published in a subsequent paper. It is already a well-known fact, however, that adequate phosphates hasten the maturity of the ears.

The mobility of the iron and aluminum compounds seems to be associated with a high sap acidity, according to preliminary tests upon normal and diseased stalks. The sap is distinctly more acid in the normal stalks than in those in which the nodal accumulations of the metallic compounds have taken place. Whether or not the decreased acidity is the result of a greater metal content of the stalks has not been learned.

#### EFFECTS OF SOIL TREATMENTS UPON THE DESTRUCTIVENESS OF THE ROOTROTS

In Plates 4, 12, A, and 13 the variations in the effects of the available metal salts are seen. The tall, vigorous plants in Plate 12, A, are the more resistant to the rootrots, while the smaller, stunted plants are susceptible and the roots are actually being rotted. It is presumed that these stunted plants have grown from infected seed, yet when infected kernels from the same ears are planted in different types of soil the percentage of damage due to the rootrot is greater in those soils which have the larger amounts of available aluminum.

Marked increases in yield of corn plants have been obtained by various soil treatments. The investigations of Abbott, Conner, and Smalley (17), Ruprecht (19), Hartwell and Pember (20), and Mirasol (28) upon different crops show that the addition of lime and phosphorus to the soil and to culture solutions has a beneficial action upon available toxic aluminum salts.

Soil of the same type, as in the field shown in Plate 4, B, was used in the pot culture experiment illustrated in Plate 14, A. Note that limestone was beneficial in producing more vigorous stalks, whereas the addition of aluminum chlorid at the rate of 500 pounds per acre caused the plants to become very susceptible to the rootrots. The limestone treatment of the Hancock County soil, Plate 14, B, was ineffective, whereas acid phosphate produced better plants. The aluminum-chlorid treatment was more harmful than in the Shelby County soil.

In 1918, an experiment conducted at Shelbyville, Ind., showed that the average yield of plants grown from nine healthy ears was increased 35.7 per cent in a soil with a higher lime content, and the average yield of plants grown from five diseased ears grown in ear rows alternating with those from the healthy ears was increased 62.2 per cent.

Results obtained by the Macon County (Illinois) Farm Bureau (27) in experiments on the control of corn rootrot by the use of fertilizers and limestone show that when "badly diseased" dent is planted the use of 3 tons of limestone per acre increased the yield of sound corn from 26.07 bushels to 44.43 bushels, or 70 per cent; from 40.13 bushels to 53.98 bushels, or 34 per cent; from 43.37 bushels to 52.90 bushels, or 21 per cent, in plots planted with different strains. The "disease-free" controls yielded at the rate of 49.69 bushels of sound corn in the untreated plot and 60.36 bushels in the treated plot, giving a 21 per cent increase. It should be noted that the "disease-free" corn yielded the highest in both the treated and untreated plots, yet the disease was most destructive in the untreated plots, all of which emphasizes the fact that the highest yields will be obtained when the best seed is planted in soil in the best state of fertility.

Other results reported in various publications have been summarized by Slipper (20) and show increases in corn yields due to the applications of lime, but no experiments have been noted which refer to the specific effects on the yield of plants growing from diseased seed.

#### GENERAL APPEARANCE OF DISEASED PLANTS

The symptoms associated with rootrots in dent have been described by Hoffer and Holbert (12), in sweetcorn by Hoffer (11), and later and in more detail by Holbert and Hoffer (13). These symptoms attributed to rootrots vary considerably because of the wide variations which occur in the type of growth of corn plants. Some strains are hereditarily weaker than others, and, as has already been said, it is believed they possess marked differences in their absorption rates of available salts. No constantly uniform symptoms have been described in open-pollinated corn because of these varying tendencies in the behavior of these heterozygous individuals.

Typical cases of individual plants affected by rootrots are shown in Plates 2, B, and 5, B. Plate 13, A, shows the disease as it occurred in a field in Shelby County in 1918. The stalks had grown to full size, but



shortly after the ears began to develop the roots became severely rotted and many of the stalks fell over. A similar condition of a field of Stowell's Evergreen Sweetcorn is shown in Plate 13, B. Here, too, many of the stalks grew to a good size but after tasseling became badly rootrotted. More of the stalks in this field were of stunted growth than in the Shelby County field, however.

These two cases are characteristic of the occurrence of the rootrots in soils high in organic matter with abundant nitrates but with little calcium and available phosphorus. The nitrates in these soils favor abundant vegetative growth of the plants. The plants under these conditions show discolorations of the nodal tissues very early in their growth, and when they reach the tassel and ear stage of development the nodal tissues frequently are much disintegrated. This disintegration of the nodal tissues results in broken shanks and a weakened root system. The translocation of foods to the roots is retarded and they cease functioning, as shown in Plate 8, B.

The general condition of the plants shown in Plate 4, A, is a very common one in large areas in the Corn Belt. The plants are stunted in their growth, and when they are cut longitudinally and examined internally the nodal discolorations will be found in abundance in the basal portion. The soils in these fields were distinctly acid, relatively low in organic matter, and deficient in calcium and available phosphorus. After the application of 3 tons of limestone to the soil in the field shown in Plate 4, A, the plants grew very well the following season, 1920. The plants in the treated plots were resistant to the rootrots which affected those in the untreated plots.

The plants more resistant to the rootrots grow in soils containing abundant nitrates and adequate calcium and available phosphorus. Calcium carbonate was found to be harmful when applied alone to the soil in the field shown in Plate 15, A. In this field acid phosphate gave the best results. The plants in the calcium-carbonate plot were more severely affected by the nodal accumulations of metals than those in the control plot.

Reference has already been made to the results of the Macon County Farm Bureau experiments (21). The rootrot phenomena which develop in plants in that locality are very similar to those shown in Plates 4, B, and 13, B. By the addition of lime and phosphate to this type of soil greater resistance to these rootrots is developed in the corn plants.

It seems from these observations that the quantitative calcium-nitrogen relations which exist in the soils are probably important in connection with the development of the symptoms of rootrots. Whether or not the high nitrogen influences the growth of the plants so that they become susceptible to the rootrots or whether the available metals, which are more abundant under the conditions of high nitrogen and low calcium in the soils, determine the susceptibility of the plants to the rootrots has not been learned. It is probably best to assume for the present that no one of these factors can be fully interpreted independently of the others, so that due consideration must be given to all of them and their interrelations established.

No detailed consideration will be given to the fertilizer experiments conducted as a part of the rootrot investigations in this paper. The results of these experiments will be published elsewhere. It is desired to refer only to the soil conditions in general where the rootrots prevail

and to show that the soil has a marked influence upon the growth of the corn plants and their relative susceptibility to the rootrots.

From the foregoing observations of field conditions under which rootrots prevail, and from the study of the results obtained in experimental plots located in various parts of the Corn Belt, the evidence seems to indicate that the soil environment determines in a large measure whether the plants become severely infected or remain relatively healthy. When kernels from the same seed ears were planted in plots in Iowa, Illinois, Indiana, and Ohio the amounts of rootrot which would develop in the different ear rows was influenced by the soil and growing season more than by the organisms present in or upon the seed planted.

#### METHODS USED TO DETECT METALS IN STALKS

During the summer of 1919 it was discovered that large quantities of iron compounds, in organic combinations, were constantly present in the nodal tissues which were colored purplish brown. The microchemical method used to detect the iron compounds in these tissues consisted in placing the tissues in a strongly acidulated (HCl) 20 per cent solution of potassium thiocyanate. When the stalks were tested in the field a stellite knife was used to cut them and the solution was applied directly to the exposed stalk tissues. By this means many hundreds of experiments and observations were made upon plants in all stages of development, and it was learned that the brownish purple nodal discoloration was associated with a relatively large quantity of iron compounds which accumulate in these tissues. Invariably the plants which were stunted in their development (Pl. 4, A, B), those whose leaves became streaked with yellow or reddish purple, those with excessive firing of the leaves, those with prematurely broken shanks (Pl. 6, A, B; 16, A, B), and those which fell over early in the field, as shown in Plate 13, A and B, are the plants which constantly are characterized by discolorations and frequent disorganizations of their nodal tissues.

#### QUANTITY OF ACCUMULATIONS INFLUENCED BY SOIL TYPES

The quantities of iron and aluminum which accumulate in plants grown from kernels from the same seed ear vary according to the nature of the soil in which the plants grow. An experiment was conducted in the station greenhouse in 1919 wherein soils representing types from different counties in Indiana were used, as follows: Soil from the field shown in Plate 4, B, representative of a calcium-deficient soil in Shelby County; soil from Bartholomew County, later found to be lacking in phosphorus; and a soil from Sullivan County, which was deficient in calcium. All pots were kept under relatively uniform conditions of moisture, temperature, and light. A rich garden soil was used as a control.

Table I shows the record of growth of three stalks in each of these four soils. The sign — indicates a trace of iron present in the nodal tissues when tested chemically, × indicates a large quantity, and o indicates no trace whatever. The series of signs represent the nodal content for each node in the stalk with the lowest node in the stalk represented at the left end of the series and the uppermost node in the stalk represented at the right end.

The best plants grew in the garden soil. They were normal green in appearance and had the iron compounds uniformly distributed throughout the stalk. This occurs in stalks of good, vigorous growth. These

results are representative of others obtained by using different kinds of ears, some of which were infected before planting.

These soils, with the exception of the control, were soils in which abundant rootrots prevailed during the season of 1919 under field conditions in these representative counties. The stalks were cut longitudinally and then tested for iron compounds. At the same time the length of the stem from the growing point to the base was measured. This serves as the best index for showing relative amounts of stalk growth. The soil influence upon the distribution of iron is very marked in the different stalks, as is seen in Table I.

TABLE I.—*Distribution of iron in vascular plate tissues in the nodes of three stalks grown from seed from the same seed ear in each of four different soils, under otherwise similar conditions for growth, at La Fayette, Ind., 1919*

Source of soil.	Height of plant.	Distance from growing point to base.	Color of leaves.	Relative distribution of iron in vascular plate tissues <sup>1</sup>
	<i>Cm.</i>	<i>Cm.</i>		
Garden.....	150	30	Normal green....	— — — — —
	150	40	.....do.....	— — — — —
	120	25	.....do.....	— — — — —
	80	12	Slight yellowish green.	X X X — — — —
Shelby County....	100	16	Green.....	X X X X X — —
	90	16	Green.....	X X X X X X X X
	75	15	Yellowish green..	X — — — — o o o
Bartholomew County.....	100	20	.....do.....	X — — — — o o o o
	90	22	.....do.....	X X X X — — o o o
	95	20	.....do.....	X X X X X X — —
Sullivan County....	80	6	Normal green....	X X X X — — o
	80	4	.....do.....	X X X — o o o

<sup>1</sup> X = relatively large quantity of accumulated metals in nodes.  
 — = trace.  
 o = no trace.

There were cases, however, where the plants in some soils were apparently diseased and would not show the brownish purple discolorations of the nodal vascular plate. When tested for iron compounds no considerable quantities would be found in the nodal tissues. During 1920 the microchemical method of detecting aluminum by use of logwood<sup>1</sup> was applied, and it was then discovered that aluminum was present in these tissues in large quantities. After the usual test for iron, the aluminum test was made, and the results from the two tests usually suggested that aluminum may be equally as important or more so than the iron in its action upon the tissues. This test cleared up what appeared to be discrepancies in interpretation of the results in the field when nodal tissues would be found in stages of disintegration but without the usual amount of iron present in the tissues. The aluminum test would indicate the total quantities of both metals present in the tissues.

#### INJECTION EXPERIMENTS

Experiments were planned and carried out during the summer of 1920 to determine the rôle of aluminum in the cornstalk, and whether or

<sup>1</sup> The logwood test consists in boiling the tissue to be tested for aluminum in a saturated solution of ammonium carbonate which has been colored deep red by logwood. This test should always be made after the relative quantity and distribution of iron in the tissue has been determined by the thiocyanate test, using a single piece of tissue for each test.

not its action was comparable to that of iron. Injections of various salts into normal-appearing stalks were made and the specific effects of single salts and of salts in various combinations were observed. In all cases a hill with two similar and apparently normal stalks was selected. The stalk not treated served as a control for comparison with the treated stalk.

#### METHOD OF INJECTING THE SOLUTIONS

The method which was used to introduce the chemicals into the plants for the study of their specific effects on the plant tissues was to insert a straight calcium-chlorid tube into the stalk just above the first node. The stalk was first punctured with a sharp cork borer of the same size as the small end of the tube. The tubes were inserted approximately to the center of the stalk and then slightly exerted to form a small reservoir for the solutions so that they could be absorbed by some of the severed vascular bundles. Measured quantities of the test solutions were then placed in the tubes, and a stopper was placed loosely into the distal end. The method is illustrated in Plate 12, B.

When the cortical tissues of the stalk were imperfectly cut the solutions would leak out, and in such cases the results were ignored. Only those cases where definite absorption of the solutions occurred have been considered. The quantities of the solutions which remained in the tubes at the end of the experiment were measured, and the total amounts were recorded. The quantities which were absorbed varied considerably for different solutes and the moisture content of the soil.

Because of the wide range in the sizes of the plants which were treated and because of their heterozygous conditions also, no accurate quantitative studies have been considered worth while yet with the strains of corn available for study. Only plants which were apparently normal were used, and each group of plants experimented upon had their origin from the same seed ear. This tended to reduce the strain differences to a minimum.

The injections were begun just at the time the plants began to tassel. They are difficult to make prior to this time because the cortical tissues are not usually mature enough to support the tubes rigidly. The relative proportion of the amount of stalk material to the quantity of solute injected can not be accurately determined because the specific effects on the tissues of varying quantities of the solute are dependent upon a definite time relation. This, too, is affected by changing plant-volume and absorption-rate entities during the time when injections are made.

The metals which accumulate in the plants under normal seasonal conditions are absorbed by the plants in very dilute concentrations from the soil solution. The concentration of the metallic salts in the soil solution is therefore a most important factor in determining the length of time required to produce the specific effects of the metals. This difference in availability of these salts is often observed in fields within small areas. In Plate 17 the effects are shown on plants of dent and sweetcorn, respectively, grown from seed taken from the same seed ears. The plants grew within 12 feet of each other, the stunted plants being taken from the ends of the rows in the soil with larger quantities of available metals. Sweetcorn is markedly more affected by these soil conditions than dent. This has been observed many times in districts where both kinds are extensively grown.

For the reasons discussed, relatively high concentrations of some of the solutions were used. It should be indicated that the quantities of injected solutions absorbed are relatively small in comparison with the quantities of the soil solution and other solutes which are absorbed by the plants. Neither is the quantitative complex of nutrients in the cell sap known. The injected solutions represent additions to the actual quantities absorbed by the plants. Obviously, it is difficult to study the effects of the various solutes in the stalks in the field. Therefore, the chief value of the injection experiments was to learn what effect the various ions had upon the plant tissues when injected into them in addition to those which were already present in the sap.

The results of the injections are given in Table II. The solutions were mostly 1 per cent concentrations of the various solutes. The characteristic effects of the iron and aluminum salts are especially noteworthy. The effects are referred to in Table II as being either injurious or causing no injury but their specific actions will be described in greater detail in the text.

TABLE II.—Effects of solutes injected into corn plants, based on the average results from five similarly treated stalks, all of the same age and approximate size, from readings made seven days after injection

Solute.	Strength.	Average quantity injected	Result.
	Per cent.	Cc.	
Aluminum chlorid.	1	5 10 5	Completely fired and streaked; nodes destroyed. Leaves streaked, partially fired; node destroyed. Do.
Aluminum nitrate.	1	10 15	
Calcium nitrate.	1	20	
Aluminum nitrate.	1	10	No apparent injury.
Calcium nitrate.	1	15	No apparent injury to leaves; nodes slightly discolored. Leaves streaked; necrosis of intervascular tissues; nodes slightly disintegrated. Streaked; necrosis of intervascular leaf tissues; and disintegration of nodal plate tissues.
Aluminum nitrate.	1	12½	
Calcium nitrate.	1	12½	
Aluminum nitrate.	1	15	Plant killed; nodal tissues destroyed. Plant apparently normal. No apparent injury.
Calcium nitrate.	1	10	
Sulphuric acid.	N/5	30	
	N/10	30	Do.
	N/20	30	Do.
Hydrochloric acid.	N/20	30	Do.
Phosphoric acid.	1	200	Do.
Tartaric acid.	1	10	Do.
Citric acid.	1	20	Do.
Acetic acid.	1	30	Do.
Malic acid.	1	30	Do.
Formic acid.	1	30	Plant killed, nodal plate tissues destroyed.
	0.25	30	Very slight marginal firing.
	0.5	30	Plant killed; nodal tissues brown
Iron citrate.	1	30	Do.
	5	30	Do.
Ferrous sulphate.	1	30	Leaves purple-streaked, the streaked tissues firing with a distinct gray color.
Iron citrate.	1	5	No apparent injury.
Magnesium acetate.	1	20	
Iron citrate.	1	10	
Magnesium acetate.	1	15	Leaves purple streaked; nodes discolored.
Iron citrate.	1	5	
Calcium citrate.	1	20	
Iron citrate.	1	12½	No injury.
Calcium acetate.	1	12½	
	1	12½	Purple streaks on leaves; nodes discolored.

Aluminum-chlorid, aluminum-nitrate (Pl. 11), iron-citrate (Pl. 18, A), and ferrous-sulphate injections (Pl. 18, B) caused marked injurious effects upon the vascular plate tissues, while iron nitrate (Pl. 18, C) and iron chlorid (Pl. 18, D) accumulated in the vascular plate tissues without producing any marked injury to either the leaves or vascular plates. These accumulations were detected by testing the tissues chemically.

Acetic, citric, tartaric, phosphoric, and malic acids in 1 per cent concentrations caused no injurious effects that could be noticed in the plants. In fact, the plants receiving the malic and phosphoric acids appeared greener and healthier than the control plants. Formic acid, however, proved to be harmful, the plants dying within two days. The rôle of the organic acids in relation to the availability of the metallic salts is being investigated further. Mazé (16) reports that malic acid is secreted by the roots of the corn plants and has an important bearing upon the absorption of the plant nutrients.

The chief purpose of the injection studies was to determine the specific effects of the iron and aluminum salts. The effect of aluminum nitrate upon the corn plant tissues is shown in Plate 11, A and B. The ultimate effect upon the leaf tissues consists of a killing of the intervascular leaf tissues and a destruction of the nodal vascular plate tissues. The progress of this action on these tissues is of much interest because the symptoms of various stages resemble closely certain of the symptoms which become prominent during the progress of development of the injuries in the stalk and leaves associated with the rootrot disease in soils with available aluminum salts.

Of a 1 per cent solution of aluminum nitrate 5 c. c. was sufficient to cause the effects shown in Plate 11, A. The quantity of aluminum ions was less than 9 mgm. It is seen then that a sudden increase in the aluminum-ion content of the stalk is disastrous and that the quantity that is necessary is exceedingly small.

Aluminum chlorid is also very toxic, but in this case the action of chlorine ions may have played a part in the injuries. These effects will be studied further. In this relation the effects of  $N/20$  hydrochloric acid should be noted. No injury to the nodal plate tissues resulted from the introduction of 30 c. c. of the acid. From these results it is evident that the aluminum ions are the toxic ones.

When  $N/100$  aluminum nitrate was injected into the stalks the injury to the leaf tissues developed slowly, the first effect being a slight yellowing of the intervascular tissues at the distal end of the leaves. The yellowing of the tissues was followed by a complete disappearance of the chlorophyll and this by a water-soaked appearance of the tissues. The affected tissues then became dry and brown, and the leaves presented the streaked appearance shown in Plate 11, B. The stages in the progress of the injury to the nodal tissues were observed in a number of plants. The accumulation was most pronounced in the vascular plate tissues, although the aluminum salts combine with the cell contents of the phloem cells along the entire vascular bundles through which they are being conducted. The vascular plate tissues (zone B in Pl. 3, A) first become yellowish brown in color, then brown, and with the deepening of the brown color the disintegration of the tissues can be noted. The advanced stages of this disintegration are shown in Plate 11, A.

## SOIL CONDITIONS WHICH FAVOR ALUMINUM AVAILABILITY

The conditions which develop in a soil to make the aluminum salts available also account for increased quantities of available iron salts, and, therefore, salts of both elements are absorbed at the same time. Because of this close chemical relation of these two metals it is necessary to study much further the effects of fertilizers and soil acidity upon their respective availabilities to the corn plants. The fact that chemical tests of diseased and normal stalks in the field show variations in the accumulations of compounds of each metal in the stalks in the nodal tissues suggests that the specific effects of the metals upon the discoloration and rate of disintegration of the vascular plate tissues may be determined by certain conditions in the soil or by variations in the "elective" capacities of different plants to absorb quantities of these metallic salts when available.

It is important, therefore, that the corn plants in the seedling and juvenile stages of growth should be least affected by the available metals. Acid phosphate applied to the soil at planting time greatly stimulates the growth of young plants in experimental plots. Whether this is due to the increased phosphorus or to the precipitation of the available aluminum which might affect the plants is uncertain, yet it is known that aluminum toxicity takes place in the absence of sufficient phosphates to precipitate the aluminum salts in the soil solution. And, furthermore, the plants in the youngest stage of development would be most rapidly affected and therefore show the effects of toxicity in retarded growth.

## TISSUES IN WHICH THE METALS ACCUMULATE

By treating the vascular plate, zone B tissues, in healthy plants with concentrated nitric acid, the cells where the accumulations of the organic metallic compounds occur first will give a distinct xanthoproteic reaction. The cells lie close to the xylem elements of the branching bundles in the vascular plate. When the disintegration of the tissues begins, these cells are the first ones to break down.

This disintegration effect on the zone B tissues may have much significance although it must be admitted that very little is known regarding it at the present time. When the metals accumulate in these nodal tissues after their injection into the stalks it has been found that the nodal tissues in zone B, as shown in plate 3, A, give a strong test for peroxidase with gum guaiacum. This reaction is probably of much significance and may indicate in a measure the probable cause of the discolorations which are consequent to the increased metallic content of these tissues.

Bayliss (3) reports that—

a peroxidase is in all probability a peculiarly active form of the colloidal hydroxides of manganese, iron, or copper preserved in this active state by the presence of an emulsoid colloid, such as gum or albumin.

He states further that the metals found to be active as peroxidases are those capable of existing in two states of different valencies. If this conclusion of Bayliss is correct it would assist in interpreting the phenomena observed in the nodal vascular plate tissues in the treated stalks and in diseased stalks. The fact that the iron and aluminum accumulations are organic aggregates of some kind in these tissues, and the fact that the tissues become strongly active enzymatically, with special reference to peroxidase, may be significant. The intensity of this action probably would determine the period of functioning of these tissues. The

increased peroxidase activity in the tissues may yield hydrogen peroxid, or other by-products, which in themselves may become toxic and account for the destruction of the tissues consequent to the metal accumulation. Furthermore, other metals and organic substances may enter the plant and stimulate this peroxidase activity in the zone B tissues. After the basal tissues and roots begin to rot the decomposition products from these rotted parts may be absorbed and act as toxins in the plant. These suggestions are given because the resultant effects of the root and stalk rots upon the plant present a complexity of phenomena which are difficult to analyze.

On the other hand, the fact that the metals accumulate in the cells giving a strong xanthoproteic reaction may confirm the report of Szűcs (24) that aluminum combines directly with protoplasm and "sets it." Loew (15) infers that calcium-protein compounds exist in the organized particles from which the nucleus and chlorophyll bodies are built up. The replacement of the calcium by another element would lead to a disturbance in the structure of the protein molecule, and this would prove fatal.

Whether or not calcium-protein aggregates exist in the cells in zone B tissues as well as in the leaf tissues, it is of much significance that when calcium was introduced along with the aluminum salts the injuries to the nodal and leaf tissues were reduced in severity during the length of time necessary for the aluminum ions alone and in equivalent concentrations to become actively toxic. The mass effect of a preponderance of calcium in the cell tissues may inhibit the disorganization of the protein aggregates by the aluminum and iron salts.

The iron-citrate solution was absorbed readily by the stalks and killed the plants within three days. The characteristic effect of the iron citrate was a rapid wilting of the stalk and a heavy precipitation of iron compounds all along the vascular bundles, as shown in Plate 18, A. The leaves developed a vinaceous gray color, and the tissues next to the vascular bundles in the stalk and leaves became brown.

Ferrous sulphate was probably the most actively toxic form of iron salts tested (Pl. 18, B). The dark brown discolorations along the vascular bundles in the leaves are characteristic of the first effects of ferrous sulphate, and this is followed by a wilting and the death of the leaves. The vascular bundles in the stalk become brown, and in zone B disintegration of the tissues occurs. The color of the darkened tissues is a brownish purple, not unlike the discolorations found in stalks growing in acid soils, as shown in Plates 2, B, and 5, A and B. The disintegration of the tissues, of course, is much more rapid than that which occurs naturally in the field, due to the stronger concentrations of the solution used. The most abundant nodal discolorations do not appear in the stalks until tassel time, a period of probably 60 to 80 days after the seed is planted, although seedlings growing in soils with large quantities of available aluminum and iron will show the basal tissue discolorations very early, according to their different relative absorption rates.

Because of the gradual and progressive changes which can be induced in the corn plants by the injection of dilute solutions of aluminum and iron salts and because these so closely resemble the progress of development of similar symptoms in corn plants growing in different types of soil, it is evident that the available salts of aluminum and iron in most soils are absorbed by the plants in dilute subtoxic concentrations and enter into organic combinations with various tissues in the plants. With



continued absorption of these metals the cumulative capacities of the plants seem to reach a limit, and then the organic metal aggregates in the cells begin to disintegrate. The rate of this action may be determined by the quantities of alkaline bases present in the plant sap. The fact also that the metals become "masked" or fixed in the plant tissues would probably explain their continued absorptions from the soil solution, the rate of absorption being largely determined by their relative availabilities.

#### QUANTITIES OF ALUMINUM PRESENT IN STALKS FROM DIFFERENT LOCALITIES

The percentage of ash in cornstalks varies considerably for plants grown in different parts of the country. Analysis of a limited number of plants shows that their ash contents vary from approximately 3 to 6.3 per cent. The aluminum content of the ash of the leaves also varies markedly, within a range extending from 1.75 per cent to as high as 5.06 per cent in some plants. In fact, the aluminum content of the ash of diseased corn plants obtained in 1920 from the Wanatah (Ind.) soil investigated by Abbott, Conner, and Smalley (1) was over 10 per cent.

Table III contains a summary of the analyses of the ash and aluminum contents of normal and rootrotted plants selected in different localities. The aluminum content has been constantly greater in the diseased stalks than in the normally growing ones. These differences suggest a relation between the occurrence of the rootrots and the aluminum content of the stalks.

TABLE III.—Percentage of ash and aluminum in normal and diseased stalks obtained from different localities in 1920

Locality.	Normal.			Diseased.		
	Number of stalks.	Percentage of ash.	Percentage of $Al_2O_3$ .	Number of stalks.	Percentage of ash.	Percentage of $Al_2O_3$ .
La Fayette, Ind. ....	3	3.22	1.77	11	3.51	2.80
Sullivan, Ind. ....	3	2.99	2.00	8	4.48	3.32
Amherst, Mass. ....				8	6.27	4.27
Battleground, Ind. ....	2	4.71	2.21	3	4.27	5.06

\* Diseased specimens only were received.

TABLE IV.—Total ash content and percentage of aluminum in healthy and diseased stalks of Reid Yellow Dent from experimental plot at Battleground, Ind., in 1920

	Ash.		$Al_2O_3$ .	
	Per cent.	Per cent.	Per cent.	Per cent.
8 healthy stalks .....	3.64	1.99		
21 diseased stalks .....	4.09	3.73		

Table IV gives a direct comparison between specially selected stalks of Reid Yellow Dent variety. Various types of stalks showing symptoms of rootrots were selected and checked against the normal-appearing stalks growing in the same soil with the same conditions for growth.

It has been learned that certain soils vary within a few feet, and for this reason some of the plants which became diseased may have had larger quantities of these salts for absorption. This soil condition is frequently found in many fields, as is illustrated in Plate 4, A.

Plate 17 shows two plants growing from the same seed ear. The plants were grown within 12 feet of each other. The soil in which the normal plant grew gave no reaction for available iron and aluminum, while the soil in which the stunted plant grew had a lime requirement of 1,100 pounds per acre. Plate 19 shows this marked difference in the rates of growth in a field. The tall plants were growing in a soil which gave no reaction for available iron or aluminum and also reacted basically to the extent of an equivalent of 600 pounds of lime per acre. A sample taken from the soil where the stunted plants were growing showed the presence of available aluminum and iron salts, and a lime requirement of 900 pounds per acre. The plants in this part of the field were badly rootrotted on June 30, 1921, while those growing in the better soil had no rot lesions on the roots.

The ash of normal stalks contains approximately 2 per cent of aluminum ( $Al_2O_3$ ), but when the proportion increases the pathological phenomena of stunted growth, leaf streaking, nodal-tissue discoloration and disintegration, broken shanks, and premature death of the stalks may develop. The nature of the symptoms, of course, is dependent upon the relative availability of the aluminum and iron salts in the soil and the meteorological conditions favoring the growth of the corn plants.

#### EFFECTS OF METALS ON FUNCTIONING OF VASCULAR BUNDLES

Many plants which wilted and died prematurely have been studied. When stalks in various stages of disease are cut near the base and placed immediately in tap water colored with methylene blue it will be found that a number of the vascular bundles are not functioning. Plate 20, A-C, and Plate 1, B, show longitudinal and cross sections through a diseased stalk. Note the small number of functioning bundles as contrasted with sections through a similarly treated normal-appearing stalk, as shown in Plate 21, A and B, and in Plate 1, A. It has been found that the central bundles in the stalk cease functioning first, and when longitudinal sections are made through the stalks and tested chemically for iron and aluminum, these are the ones which give the reactions for the largest quantities of accumulated metals in the vascular plate tissues.

This plugging of the vascular bundles is a common effect of various solutions of the metals when absorbed by corn plants. Aluminum salts are especially active in this regard. It has been found in experiments that plants wilt very rapidly when cut and placed in  $N/100$  solution of aluminum nitrate, whereas in the same concentration of ferrous sulphate larger quantities are absorbed before wilting begins. This latter type of wilting, due to ferrous sulphate, is subsequent to marked leaf-tissue injuries, whereas the wilting due to the aluminum usually precedes the leaf-tissue injuries. The physiological-chemical interpretation of this action is not known. Neither is the physiological effect of the metal salts upon the phloem tissues known. The changes which occur in plants with large quantities of metals in their tissues offer many problems which must be solved according to their influence upon the functions of the different tissues, such as absorption, transpiration, translocation,

and general metabolism. It is known that they act in a beneficial manner upon certain tissues when present in very small quantities. When the quantities are supra-optimal the effects become pathological.

The influence of the metals which frequently accumulate in the scutellum of the kernels, as shown in Plate 5, C, upon germination and growth of the seedlings must also be considered. There is some evidence to indicate that they affect the amylase activity in these tissues and inhibit the growth of the young seedlings.

Many weakly germinating seedlings have been studied in this regard, and it has been found that many seedlings which possess a darkened scutellum, such as has been described by Adams and Russell (2), will show relatively large quantities of accumulated iron in these tissues in comparison with the quantities which normally are present in the seedlings of high-yielding, disease-free types. Whether or not these tissues are affected by the metals so that they too become more easily invaded by fungi has not been determined, yet they frequently are infected by *Rhizopus nigricans* Ehrh., *Penicillium* spp., and other organisms.

The conditions of high aluminum availability in acid soils are chiefly those which occur when calcium and phosphorus salts are lacking, as shown by Mirasol (18). For this reason the effects of aluminum compounds may rapidly become injurious, due perhaps both to a cumulative toxicity in the concentration centers in the plants and to a phosphorus starvation of the growing and functioning tissues.

The influence of nitrates in the soil has already been referred to. When they are abundant the plants seem to grow more vigorously and the quantity of aluminum compounds may not reach toxic proportions in any particular part until the plants have made good vegetative growth. When the available nitrates, as well as the calcium and phosphorus in the soil, are low, as shown in Plate 4, A and B, and Plate 19, the cumulative aluminum toxicity develops rapidly and the growth of the plants is retarded. The nodal tissues become disorganized very early in the life of the plant. Plants in the condition shown in Plate 19 have recovered from this stunting effect after heavy rains. New roots may push out, provided the basal tissues are not too badly affected, and with a decreased percentage of available salts of aluminum and iron in the soil more rapid growth may take place. Any retardation of the early growth of the stalks due to any cause, either the accumulated metals, infected seed, or insect injury, results in a lessened productive capacity of the plant.

#### IMPORTANCE OF AVAILABLE PHOSPHATES

As the quantities of available aluminum compounds vary according to the type of the soil and according to the farm management practiced upon the different soils, and, furthermore, as the amount of moisture affects the quantities of available aluminum compounds, as has been shown by Conner (6), it is evident that the rate of absorption of these salts must be related to their respective availabilities during different periods of the growing season. The other available elements, calcium and phosphorus, also vary in quantity in the different soils. The phosphorus relation is the most important one in connection with the aluminum and iron salt availabilities. The aluminum salts are not available in any considerable quantities in a soil containing soluble phosphates. It is a recognized fact that most acid soils are deficient in phosphates.

The greater the intensity of the acidity the greater are the quantities of aluminum and iron salts which become available.

When lime is added to acid soils it is believed that the conditions which operate to make the aluminum and iron compounds available are destroyed, and even though aluminum salts may still be available after calcium carbonate is added, the addition of soluble phosphates will rapidly precipitate the aluminum salts and render the aluminum inert so far as absorption by corn plants is concerned.

Lime has a beneficial effect according to Hartwell and Pember (10), Mirasol (18), and Conner (7), although its action is markedly slower than that of acid phosphate.

Aluminum phosphate is relatively insoluble, yet when used as a fertilizer it produces an increased growth of corn plants, according to unpublished results obtained by Conner. This is perhaps due to the hydrolysis of the aluminum phosphate, as shown by Cameron and Bell (4), the result of which would yield inert aluminum hydroxid and available phosphorus to the plant.

These statements are based upon studies of plants in fertilizer experiments in the field and greenhouse.

#### DISCUSSION

The rôle of phosphorus in plant growth and reproduction is a problem which has been worked upon by many investigators, and its relation to certain of the metabolic processes is fairly well understood. The ash content of kernels of corn averages 45 per cent phosphoric acid. Its importance, therefore, in the production of seed corn needs no further emphasis.

The fact that phosphorus is deficient in most acid soils is also well known and needs no discussion. The importance of available aluminum compounds in the soil, however, is just beginning to be studied intensively, and the fact that when the aluminum salts are available or become available the supply of available phosphates is not adequate for the needs of plants in normal growth is one which presents a complex problem for investigation.

The rôle of lime in the soil in relation to the conditions under which aluminum and iron salts become available for absorption is probably that of a stabilizer of the soil reaction. The hydrogen-ion index of a black loam soil deficient in phosphates and lime varied markedly during the growing season of 1920. Therefore, if the soil reaction can be maintained constantly near neutrality the quantities of available aluminum and iron salts will be less for absorption by the corn plants.

The growth phenomena of the corn plants can not be normal under such conditions of deficient phosphates and lime and available aluminum and iron salts. It is necessary, therefore, to isolate the phenomena due to specific ions, if possible, and study them separately. This was attempted in part by injecting solutions of supposedly harmful salts and others into normal plants. By this means it was found that aluminum and iron ions were harmful and would produce effects similar to those which become manifest in many stalks which are affected by rootrots. A specific toxic action was stimulated by the aluminum and iron ions, respectively. Organic and mineral acids injected into the stalks in higher concentrations than are ever found in acid soils produced no specific results. Furthermore, when calcium ions were introduced in

large quantities the intensity of the action of the metal ions seemed to be inhibited during the time usually required in the experiments for the aluminum and iron ions to become injurious.

These injection experiments have their shortcomings. The salts absorbed naturally by the plants, as well as the quantity of water, are unknown quantities. For this reason the concentration of the specific ions after injection into the plant could not be approximated. The resultant effects of introducing definite quantities of solutes irrespective of their dilution were studied. By this means the cumulative toxicity of specific aluminum and iron ions was definitely proved, although the toxic and lethal concentration within the stalks are unknown. Probably the aluminum and iron ratio to the green weight of the plants offers the best basis for comparison, but this must be definitely established. With a large quantity of available metals during the seedling stage the ratio would be very high, but with rapid growth of the plants under favorable meteorological conditions and abundant nitrates the ratio may be kept relatively low until later in the life of the plant. Furthermore, different plants will absorb the metals at different rates, and this absorption-rate capacity of the plants also complicates the problem.

The brownish-purple discolorations and frequent disintegrations of the nodal tissues are the result of the metal accumulations in these tissues. Because of the soil relations which have just been described, it seems reasonable to conclude that when the accumulations of metal, especially aluminum, occur in abundance in the corn plants, the soil is deficient in available phosphates. Furthermore, under these conditions if the organisms commonly found in rotted roots, such as *Fusarium moniliforme*, *Trichoderma* sp., *Rhizopus* spp., *Penicillium* spp., a white bacterium, and others, are present in the soil they will produce definite rot lesions in the roots and basal parts of the stalks. The condition of the tissues within the plants seems to determine the rate of development and consequent injuriousness of these rots.

Resistance to the rootrots in corn plants seems to be defined (1) by the respective abilities (inherited) of the plants to absorb materials present in the soil solution and (2) by the relative concentrations of deleterious ions presented to the roots in the soil solution.

In this consideration of the rootrots of corn it should be borne in mind that the importance of using seed which has not been damaged by infection by fungi and bacteria is in no way diminished. The value of using seed which has not been weakened by infections has already been indicated (11, 12, 13). These investigations cover those effects upon plants which occur irrespective of the seed infections and which are more directly referable to the soil influences upon the growth of the plants and their relative resistances to these influences which determine susceptibility to and the extent of development of the rots in the plant parts. If these infections can be controlled in a large measure by soil treatments and by breeding better strains of corn, the practical importance of these investigations will be established. Therefore, seed ears should be selected which are free from infections and brownish purple discolorations of the shank tissues. Moreover, the plants in the field which do not show accumulations of large quantities of the metals, as evidenced by the nodal plate discolorations and disintegrations of the tissues, by the time the ears are matured, are likely to be of greater value to the corn breeder than those plants which show these characteristics when grown under similar conditions. These statements apply

equally well to plants in all localities, irrespective of whether rootrots develop in them and appear to become a serious menace to the crop.

#### SUMMARY

(1) One of the most characteristic differences between normally growing corn plants and those which become severely rootrotted is the condition of the vascular plate tissues in the nodes of the stalks. The plants which become severely rootrotted are those which have the nodal tissues discolored and in various stages of disintegration.

(2) This disintegration of the nodal plate tissues begins in the absence of any specific organisms in the tissues.

(3) The brown, yellowish brown, and brownish purple discolorations with their consequent disintegrations which are frequently found in diseased plants have been produced artificially by injecting solutions of certain salts of aluminum and iron into the plants. Definite chlorophyll and leaf-tissue changes have been produced also. Other factors, however, may operate to produce similar effects.

(4) These artificially induced changes in the plant parts closely resemble the phenomena which develop in plants growing in the field under conditions favorable to rootrots.

(5) The most severe cases of rootrots have been found in soils notable because of their deficiencies of lime and available phosphates.

(6) Such soils have variable quantities of salts of aluminum and iron available for absorption by plants.

(7) Corn plants show marked differences in the quantities of aluminum and iron salts which are absorbed by them. These differences develop when the salts are available in subtoxic concentrations in the soil and are believed to be due to specific selective capacities of different plants to absorb the available aluminum and iron salts from the soil. This type of selective absorption cannot operate when the aluminum and iron salts occur in quantities which are toxic to the roots.

(8) A definite cumulative toxicity of aluminum salts within the plants was established by the injection experiments, and it is believed that the same phenomenon occurs naturally in the field. The relative quantities of the available metals and of nitrates in the soil determine, in a large measure, the rate of development of the cumulative toxicity of the metals within the plants. Those plants which contain the largest quantities of these metals are the ones which seem to develop the most severe cases of rootrots when the organisms are present in the soil and the meteorological conditions favor their optimum growth.

(9) When abundant aluminum injuries occur in the corn plants in certain fields it is an indication that the soil is deficient in available phosphates.

(10) The application of lime and phosphates to soils in which rootrots have developed in destructive proportions has been decidedly beneficial in controlling them. The use of limestone alone in some instances proved harmful, but in all cases studied so far the application of available phosphates produced plants which were better and more resistant to the rootrots.

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PLATE 1

A.—Longitudinal section through a node of a normal stalk. Most of the bundles were functioning, as is indicated by their staining with methylene blue. Very few bundles tested for iron and aluminum in zone B.

B.—Longitudinal section through a node of a diseased stalk. Very few of the bundles were functioning. The inactive bundles contained large accumulations of iron (red) and aluminum in the zone B region. This is characteristic of stalks showing symptoms of rootrots.

## PRODUCTION OF A GROWTH-PROMOTING SUBSTANCE BY AZOTOBACTER<sup>1</sup>

By O. W. HUNTER

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It is maintained by many investigators that the animal cell is incapable of synthesizing vitamins and that such cells are thus required to obtain their food accessories from the vegetable kingdom. If this is true, it can logically be asked, do plants need such growth promoting factors? If so, do they synthesize them or from what source are they obtained?

Bottomley's (2)<sup>2</sup> experiments on peat led him to state that the growth of plants require food accessories or auximones. He grew aerobic soil organisms in peat for several days. This was then sterilized, inoculated with *Azotobacter* and incubated. As a result there were produced in the peat substances which greatly stimulated plant growth. Similar results were obtained by Mockenridge (4) and Jones (3).

The application of the vitamin theory to microbial nutrition is now prevalent. While there has been a large amount of attention focused upon the need of food accessory factors for the normal growth of microorganisms, but little thought has been placed upon the vitamin content of microorganisms or their ability to synthesize such, regardless of the fact that one of the most abundant known supplies of the so-called water-soluble B vitamin is found in a one-celled organism, the yeast. This vitamin content of yeast was first reported by Funk (1).

Pacini and Russell (5), from their experiments concluded that *Bacillus typhosus* Eberth-Gaffky can manufacture vitamins and can likewise stimulate growth of the animal cell. They grew *B. typhosus* in a synthetic medium, the bacterial cells from which were collected and extracted with alcohol. The alcoholic extract was evaporated down to dryness and the residue dissolved in water. This extract was used in their feeding experiments on white rats.

Recently much attention has been directed toward the use of yeast for the quantitative measurement of the vitamin content of test substances, the principle of which is dependent upon the assumption that the yeast needs a food accessory factor for its growth in an inorganic synthetic medium. This accessory factor is considered identical with the water-soluble B vitamin.

Experiments to determine the reliability of the test appear to have brought forth some interesting facts. This dependence of yeasts upon the antineuritic vitamin is doubted by MacDonald and McCullom (7).

<sup>1</sup> Accepted for publication Jan. 16, 1922. Contribution No. 39 from the bacteriological laboratories of the Kansas Agricultural Experiment Station, reporting in part the results of project No. 138.

<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 811.

They cultivated yeast in a synthetic medium containing no possible source of vitamine and obtained a good growth. They concluded that the yeast must either synthesize its own vitamine or grow without such "bios." Nelson, Fulmer, and Cessna (6) likewise grew yeast in a synthetic medium and concluded that yeast could synthesize its own vitamine.

The controversy over the vitaminic needs of yeast stimulated the author's interest in the *Azotobacter* cell. The ability of this organism to grow rapidly and vigorously in a synthetic medium containing no nitrogen is familiar. Such a medium contains no possible source of vitamins, if care is exercised in its preparation. Media thus prepared in this laboratory have always afforded a vigorous growth of *Azotobacter* when placed under favorable conditions for growth. It is assumed, therefore, that the *Azotobacter* organism is not dependent upon such growth-promoting factors for development or that it is capable of synthesizing vitamins for its needs.

Under these conditions it would be of interest to learn whether this growth resulting from *Azotobacter* development contained any vitamins. Their presence would thus indicate the ability of *Azotobacter* to synthesize growth promoting substances.

Preliminary feeding experiments on white rats, with a diet in which the water-soluble B vitamine was replaced by dried *Azotobacter* cells, revealed the possible presence of a growth promoting substance. In this experiment the *Azotobacter* was grown on dextrose Ashby agar placed in large wooden trays to give a large surface exposure. The growth was scraped from the surface of the agar after two days' incubation at 30° C. and air dried.

Three white rats were fed on the following diet:

	Per cent.
Starch.....	58
Casein.....	20
Lard.....	10
Butter.....	5
Ash.....	3
Agar.....	2
<i>Azotobacter</i> .....	2

The beneficial effects of the feed are shown in figure 1:

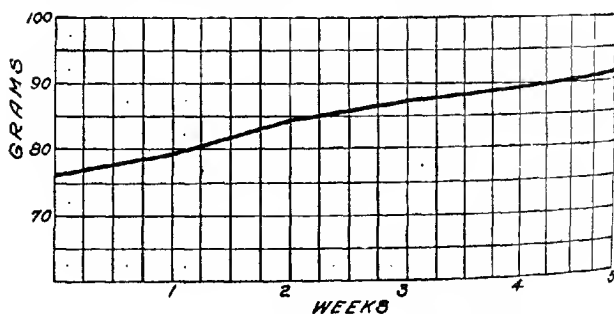


FIG. 1.—Average gain for three rats fed *Azotobacter*. Control rats died.

Three rats fed on the same diet minus the *Azotobacter* failed to gain in weight, developed the characteristic sore eyes, and soon died.

These results indicated the possibility of the presence of a food accessory factor for rats fed on a diet deficient in water-soluble B. However, needed

improvement in the medium used and in the method of cultivation were desired for further investigation. This was accomplished by devising a means for cultivating the *Azotobacter* rapidly in a large volume of liquid culture medium.

A description of the method of procedure and experimental data follows.

#### MEDIUM

To insure the absence of any known food accessory compound, chemically pure chemicals were employed in the preparation of the synthetic medium. All media were made up with a reaction of  $P_n$  7.0 to 7.4 and were thoroughly sterilized at 20 pounds pressure for 30 minutes.

The composition of the medium was as follows:

Distilled water.....	1,000.00 cc.
Potassium acid phosphate.....	.50 gm.
Magnesium sulphate.....	.20 gm.
Sodium chlorid.....	.20 gm.
Dextrose.....	10.00 gm.

#### STARTERS

Starters of pure *Azotobacter* cultures were made by inoculating flasks containing about 300 cc. of the dextrose media with a portion of the emulsion from a young dextrose Ashby slant culture. This was thoroughly aerated for two days at a temperature of 30° C. Several strains of *Azotobacter* cultures have been used in these experiments. All cultures were isolated in this laboratory from different samples of soil. No attempt has been made to differentiate the cultures as to species. Pigment production was common in all cultures used.

It was aimed to use only pure cultures of *Azotobacter*, and repeated streakings upon dextrose Ashby agar plates were made to insure freedom from contamination. Frequent morphological examinations were made to insure purity of the cultures.

#### METHOD OF CULTIVATION

A rapid multiplication of *Azotobacter* cells was encouraged by vigorous aeration of the liquid cultures. Bottles containing 2,000 cc. of sterile media were inoculated with 10 to 20 cc. of the *Azotobacter* starter. These were placed in the incubator, temperature of 30° C., and aerated by bubbling air vigorously and continually through the medium. The air was passed through the cultures by attaching the bottle equipped with Polin's aerating tubes to a vacuum system. Contamination and evaporation of the cultures were reduced to a minimum by filtering the air, first through a sterile cotton filter and then by washing in two or three flasks of sterile water. The air was thus filtered through cotton and rinsed in water before entering the culture media. From 12 to 24 of these bottles were aerated at the same time. A vigorous growth was produced within two to four days. If the culture is pure and no contamination occurs, the reaction of the media should remain close to the reaction of the media before inoculation.

It is not believed that the inoculum supplies accessory food factors for stimulating the growth of *Azotobacter* (in this synthetic medium). Ten flasks containing 200 cc. of the media were inoculated, respectively,

in duplicate with 2 cc., 1 cc., 0.5 cc., 0.1 cc., and 0.01 cc. of a young starter. All cultures were aerated at 30° C. for four days. Sugar determinations were made at the end of two and four days. All cultures exhibited a heavy growth within two days. The sugar analysis at this time showed the flasks to contain the following amounts of dextrose:

	Gm. dextrose per 100 cc.
Flasks with 2 cc. inoculum.....	0.60
Flasks with 1 cc. inoculum.....	.53
Flasks with 0.5 cc. inoculum.....	.60
Flasks with 0.1 cc. inoculum.....	.48
Flasks with 0.01 cc. inoculum.....	.53

The cultures gave only a trace of dextrose at the end of four days' incubation. The growth at this period was more vigorous in all cultures than the preceding one. It does not appear from these results that the growth of *Azotobacter* in such a synthetic medium could be attributed to stimulating factors carried over with the inoculum. The *Azotobacter* growth in the following experiments was collected by centrifuging the liquid culture in a Sharples Laboratory Super-Centrifuge. The residue was recovered and air dried.

#### FEEDING EXPERIMENTS UPON WHITE RATS

The presence of a growth-promoting substance in this dried *Azotobacter* was tested upon white rats. The dried *Azotobacter* was added to a diet deficient in water-soluble B vitamine. The composition of the feed used was as follows:

	Per cent.
Starch.....	58
Extracted casein.....	10
Tankage.....	10
Ash.....	3
Agar.....	2
Butter.....	10
Lard.....	3
<i>Azotobacter</i> .....	2

On April 28 six white rats were placed in individual pens and fed until May 24 on a regular stock food. Upon this date three of the rats were placed upon the experimental feed and three upon the same feed, except that Fleischmann's yeast was substituted for the *Azotobacter*.

The stimulating effect of each test substance is demonstrated by the curves in figure 2, plotted from the data obtained. It will be observed that during the intervals between the second and fourth weeks but little gain was recorded. This is attributed to the ash content of the feed. A new portion of feed containing new ash was prepared at this time. The rats fed on the *Azotobacter* feed show a greater comparative growth than the rats fed on the yeast diet. The average weights of the three rats fed on the *Azotobacter* feed is compared with the average weights of the three fed on the yeast food by the curves plotted in figure 2. The results from this experiment indicate that *Azotobacter* is fully as good as baker's yeast, if not better, as a growth-promoting substance for white rats fed on a diet deficient in water-soluble B.

# EXPERIMENTS WITH PIGEONS

The curative effect of Azotobacter upon pigeons affected with polyneuritis was noted. A pen of pigeons was started on a feed of polished rice and water July 14. When pronounced symptoms of polyneuritis

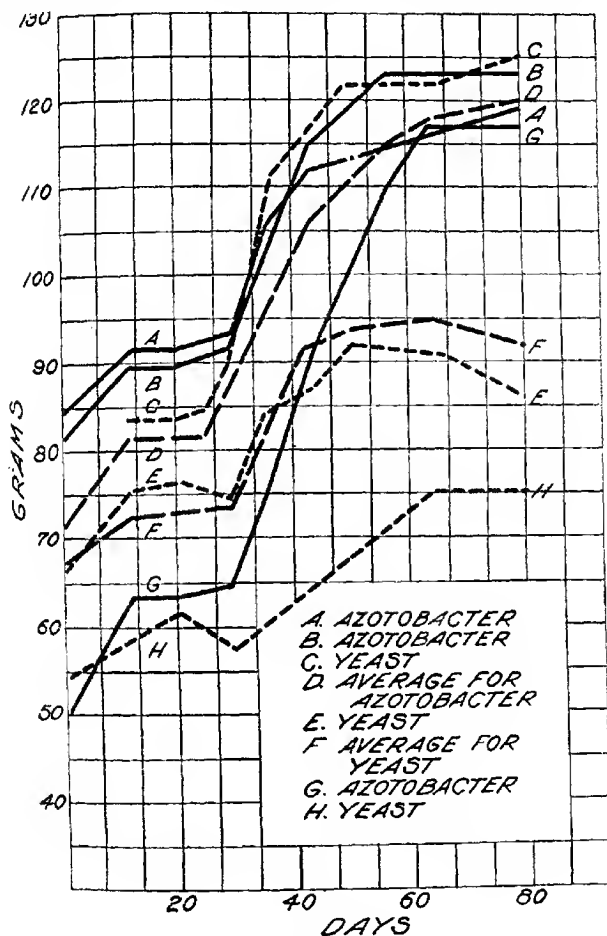


FIG. 2.—Influence of Azotobacter and yeast upon the growth of rats.

were observed, the sick pigeon was fed by hand, portions of the dried Azotobacter. The treated birds were left on the rice diet throughout the experiment. The record of observation follows:

Pigeon 3743 showed symptoms of polyneuritis on July 25. It grew daily worse and upon July 28 was exceedingly lame and unable to stand

on its feet. Upon this date it received 1 gm. of *Azotobacter* and on the following day it was up on its feet but still lame. On July 31 it was fed another gram. Its recovery was practically complete, with the exception of a slight lameness in one leg. Symptoms of polyneuritis were again evident on August 6, by lameness and inability to stand on its feet. It was fed 1 gm. of *Azotobacter* in the morning of this date. Marked improvement was shown by evening, and the next morning it was up on its feet feeding about the pen. On August 8 the pigeon appeared in normal health, with the exception of a slight lameness in one leg. The experiment was discontinued on this date and no further observations were made.

Very pronounced symptoms of polyneuritis appeared in pigeon 3742 on July 4. It was given on this date 1 gm. of *Azotobacter*. The next day it was on its feet and was given 0.5 gm. Its recovery was seemingly complete. On July 14 it again developed "limberneck" and leg weakness and died that night. This time it received no *Azotobacter*.

Pigeon 3740 developed very pronounced symptoms of polyneuritis on July 27 in the form of "limberneck" and inability to stand or walk. One gram of *Azotobacter* was given it on this date. Much improvement was noted the following day. On July 29 it received another gram and the following day 0.5 gm. Marked improvement followed, and to all outward appearances recovery was complete. It developed leg weakness but no "limberneck" the morning of August 6. At this time it received another gram of *Azotobacter*, and this dose was repeated again in the evening. The following morning it was up and feeding about the pen. No further observations were made, as the experiment was discontinued.

Pigeon 3741 developed typical polyneuritis July 25, exhibiting the characteristic "limberneck" and leg weakness. It was given on July 27, 1 gm. of *Azotobacter*. Improvement was noted the following day. It was fed another gram on July 29, although its improvement was marked, and on July 30 it received 0.5 gm. To all general appearances it was normal the next day.

That *Azotobacter* exerts a pronounced curative effect upon pigeons affected with polyneuritis seems conclusive.

#### CONCLUSIONS

- (1) If a growth-promoting substance is a requirement for *Azotobacter* development, it is capable of manufacturing such.
- (2) The *Azotobacter* can synthesize a food accessory factor similar to water-soluble B.
- (3) This food accessory factor stimulated a greater net gain in white rats than did baker's yeast.
- (4) *Azotobacter* exerts a pronounced curative effect upon pigeons affected with polyneuritis.

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## SCLEROTINIA CARUNCULOIDES, THE CAUSE OF A SERIOUS DISEASE OF THE MULBERRY (*MORUS ALBA*)<sup>1</sup>

By EUGENE A. SIEGLER, Assistant Pathologist, Office of Fruit-Disease Investigations  
and ANNA E. JENKINS, Assistant Mycologist, Office of Pathological Collections,  
Bureau of Plant Industry, United States Department of Agriculture

In 1903, Orton<sup>2</sup> described a disease of the mulberry characterized by the "peculiarly enlarged portions of the aggregated fruit." The disease was said to occur in Georgia, Alabama, and Mississippi. Dr. M. B. Waite, Pathologist in Charge, Office of Fruit Disease Investigations, Bureau of Plant Industry, states that specimens of mulberry fruits showing this condition have been received from various southern States by the United States Department of Agriculture a number of times during the past 20 years. Taubenhau<sup>3</sup> reports the occurrence of this disease in Texas, where it is known under the name of "popcorn" disease of mulberry.

In July, 1920, specimens of diseased mulberry fruits (*Morus alba* L.) were received from Scranton, S. C. Specimens of this material show the greatly enlarged condition of the ovary (Pl. 1, A), and the calyx lobes are small and nonsucculent instead of being fleshy as in the normal fruits. Upon microscopical examination the ovaries are found to be entirely filled with mycelium and all traces of host tissue are lost. The hyphae are compact, producing a typical sclerotium, and preventing the formation or further development of drupelets. A layer of sporogenous hyphae completely envelops this sclerotium and produces small hyaline spores in a compact palisade within the ovary wall (Pl. 1, B). The spores, presumably microconidia, are often exuded in immense numbers and collect in what resembles a waxy mass on the outside of the ovary wall (Pl. 1, C, a). It is interesting to note the occurrence of microconidia on the diseased fruit, as no reference to microconidia of species of *Sclerotinia* occurring other than on culture media has been found in the literature. This disease undoubtedly is the same as that reported by Orton.<sup>4</sup>

The nature of the disease led us to suspect that we had either a species of *Sphacelia*, or, more probably, the microconidial stage of the genus *Sclerotinia*. It was thought that infection probably occurred at the blooming period of the host and resulted in the formation of such a sclerotium. Accordingly on March 18, 1921, an inspection was made of the planting in South Carolina from which diseased specimens had been received in 1920. At that time apothecia (Pl. 2, A) of a fungus belonging to the genus *Sclerotinia* were found in large numbers on the ground beneath the infected trees. The apothecia were attached to the infected

<sup>1</sup> Accepted for publication Oct. 28, 1921. The technical description from page 815 of this paper was printed in *Science*, vol. 55, no. 1422, p. 353, March 31, 1922.

<sup>2</sup> ORTON, W. A. ON A FUNGUS DISEASE OF THE MULBERRY FRUIT. (Abstract.) *In* Exp. Sta. Rev., v. 14, no. 6, p. 517-517, 1903.

<sup>3</sup> TAUBENHAUS, J. J. ON A PECULIAR DISEASE OF MULBERRY FRUIT. *In* Nature Study Rev., v. 17, no. 7, p. 282-286, 3 figs., 1911.

<sup>4</sup> ORTON, W. A. *OP. CIT.*

seeds (really ovaries) which were buried at an average depth of  $\frac{1}{2}$  inch in the soil. The ascospores were found to be mature on this date and capable of germinating in tap water. A microscopical examination of the blossoms showed practically 100 per cent infection at this time. An opportunity was not afforded to inoculate healthy blooms with ascospores.

Specimens of young blooms not less than 8 feet above the nearest group of apothecia were collected on March 18, placed in a killing solution, embedded in paraffin, and later sectioned. Upon microscopical examination of the prepared mounts typical ascospores (fig. 1, A) were readily found on the pistils. The foregoing observations constitute sufficient evidence to warrant belief in the pathogenicity of the fungus.

No record of a similar disease of mulberry in this country has been found. Two somewhat similar diseases due to *Sclerotinia shiraiana* P. Henn and *Microglossum shiraianum* P. Henn have been reported in Japan. In a letter of June 20, 1921, Dr. Shirai, of the Tokyo Imperial University, states that these fungi attack—

mulberry fruits and form sclerotia just in the same manner ... and occur on the tree at the same time.

He also states that these two fungi can not be distinguished by the forms of the sclerotia but that the fruiting bodies are entirely different. Material of *S. shiraiana* was kindly sent by Dr. Shirai for comparison with the fungus collected in South Carolina. The latter differs in many respects from *S. shiraiana* and especially in its effect on the fruit and in the size and form of the ascospores. In *S. shiraiana* the entire aggregate fruit is transformed into a sclerotium (Pl. 2, B, a, b), while in our fungus the multiple fruit breaks up and the sclerotia are formed from individual druplets (Pl. 2, A, b). There is also greater variation in the size of the druplets in the American material as

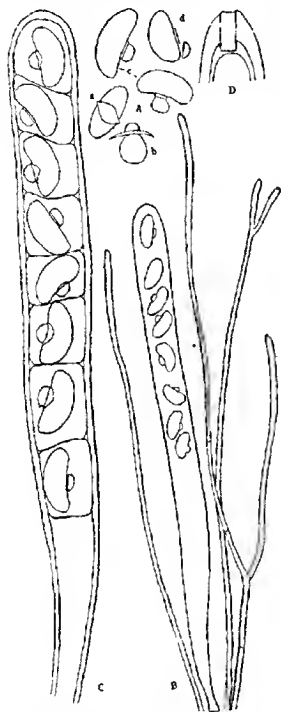


FIG. 1.—*Sclerotinia carunculoides*: A, Ascospores with bodies adjoining the concave surfaces; a, view from above showing rhombic shape of body adjacent to the spore; b, transverse section showing crescent shape of the body adjacent to spore; c, longitudinal section of spore, showing location of hemispherical body; d, spore with hemispherical body dislodged.  $\times 1,600$ . B, Ascus, a-cosporous, and paraphyses.  $\times 840$ . C, Ascus, showing ascospores surrounded by gelatinous membrane producing a cross-walled effect.  $\times 1,600$ . D, Tip of ascus.  $\times 1,600$ .

compared with the specimens from Japan (Pl. 2, B, c). A Botrytis stage develops in the fruits attacked by *S. shiraiana*, while in this country there is no record of a Botrytis on mulberry fruit. However, it is possible that a conidial form other than the microconidial may be found in this country.

In connection with the differences in the size and form of the ascospores, it should be noted that the spores of *Sclerotinia shiraiana* are elliptical and are, according to comparison with material received from

D<sup>r</sup>. Shirai, slightly larger than the spores of our fungus. The spores of the latter are reniform, and attached to the concave surface of each spore are peculiar bodies (fig. 1, A) which are striking structures morphologically. Viewed under low magnification these bodies resemble a caruncle, (fig. 1, B). Under higher magnification they are seen to be composed of two parts, one a body adjacent to the spore and more or less rhombic in shape as seen from above (fig. 1, A, a) and crescent-shaped (fig. 1, A, b) in transverse section of the spore, the second a body adjoining the first and more or less hemispherical (fig. 1, A, c). No description of similar bodies has been found in the literature on *Sclerotinia* or on closely related genera. The presence of these caruncle-like bodies made possible the identification of the ascospores on the pistils. Further studies are being made on the nature of these structures. Stained preparations show the spores to be surrounded by a gelatinous-like substance which appears to be broken up into segments in the ascus, so that the ascus has somewhat the appearance of being marked by transverse septa (fig. 1, C). Somewhat similar appearances are shown by Woronin<sup>3</sup> on Plate 10, figures 21 to 23, of his drawings of *Sclerotinia megalospora* Wor. and by Boudier<sup>4</sup> on plate 318 of his drawings of *Peziza cucullata* Boud.

As stated above, this fungus differs morphologically from *Sclerotinia shiraiana*, which produces a somewhat similar but distinct disease in Japan; it also appears distinct from any other described species. The name, *Spermatomyces mori*, gen. nov., sp. nov., which was suggested by Ortou (l. c.),<sup>7</sup> referred to the microconidial stage, and since no technical description was published, it must be considered a *nomen nudum*. The fungus is described as follows:

*Sclerotinia carunculoides*, n.sp.<sup>8</sup>

Apothecia one to several from a single sclerotium, disc cupulate to subcupulate, 4 to 12 mm. in diameter, inside snuff-brown,<sup>9</sup> outside Prout's brown; stalk cylindrical, flexuous, smooth, attenuated downward, 15 to 42 mm. in length, reaching a diameter of 1.5 mm., color Prout's brown; asci cylindrical to cylindro-clavate, 104 to 123  $\mu$  by 6.4 to 8  $\mu$ , average 117 by 7  $\mu$ , 8-spored; ascospores uniseriate, reniform, hyaline, 6.4 to 9.6  $\mu$  by 2.4 to 4  $\mu$ , average 7.6 by 3.1  $\mu$ , with two bodies on the concave surface: namely, a body more or less rhombic in shape as seen from above, 2 by 4  $\mu$ , and adjoining it, more or less hemispherical body 3  $\mu$  in its longest diameter; paraphyses filiform to cylindro-clavate, simple or branched, septate or nonseptate, 94 to 128  $\mu$  by 1.8 to 2  $\mu$ ; microconidia hyaline, subglobose, 2 to 4  $\mu$  by 2 to 3.2  $\mu$ , average 2.8 by 2.5  $\mu$ ; sclerotia black, fairly regular, subspherical with depressed surfaces.

On fruits of cultivated *Morus alba*. Type material collected at Scranton, S. C., U. S. A., March, 1921. Specimens have been deposited in the Office of Pathological Collections, Bureau of Plant Industry, United States Department of Agriculture, Washington, D. C.

<sup>3</sup> Woronin, Michael. UBER DIE SKLEROTIEN-KRANKHEIT DER VACCINIEN-BEEREN. ENTWICKELUNGSGESCHICHTE DER DIESSE KRANKHEIT VERURSACHENDEN SKLEROTINIEN. Mém. Acad. Imp. Sci. St. Pétersb. Nr. 7, t. 35, no. 8, 49 p., 10 col. pl. St. Pétersbourg, 1888.

<sup>4</sup> Boudier, Émile. ICONES MYCOLOGICAE OU ICONOGRAPHIE DES CHAMPIGNONS DE FRANCE PRINCIPALEMENT DISCOMYCETES. . . . . t. 2, pl. 318 (col.), t. 4, p. 177. Paris, 1905-1910.

<sup>7</sup> Ortou, W. A. or. CY. *Sclerotinia carunculoides*, sp. nov. Ascomatibus ex uno sclerotio solitariis vel pluribus exorientibus, discis cupulatis vel subcupulatis, 4-12 mm. diam., intus sternutamento-brunneis, extus "Prout's"-brunneis, stipitibus cylindraceis, flexuosis, elatis, dorsum attenuatis, 15-42 mm. longis, usque 1.5 mm. diam., "Prout's"-brunneis; ascis, cylindraceis vel cylindro-clavatis, 104-123  $\times$  6.4-8  $\mu$ , medio numero 117  $\times$  7  $\mu$ , octosporis: ascosporis uniseriatis, vix distincte corpusculo plano, nonnihil rhombio desuper viso 2  $\times$  4  $\mu$ ; et hoc adtingente altero corpusculo plus minusve hemisphaerico, longissimo diametro 3  $\mu$ , paraphysibus filiformibus vel cylindro-clavatis, simplicibus vel ramosis, septatis vel non-septatis, 94-128  $\times$  1.8-2  $\mu$ ; microconidiis hyalinis, subglobois, 2-4  $\times$  3-4  $\mu$ , in fructibus cultis Mori albae, Scranton, South Carolina, U. S. A.

<sup>8</sup> Ridgway, Robert. COLOR STANDARDS AND COLOR NOMENCLATURE. IV, 43 p., 53 col. pl. Washington, D. C., 1912.

<sup>9</sup> Ridgway, Robert. COLOR STANDARDS AND COLOR NOMENCLATURE. IV, 43 p., 53 col. pl. Washington, D. C., 1912.

## SUMMARY

*Sclerotinia carunculoides*, n. sp., is described as causing a disease of fruits of cultivated mulberry (*Morus alba*) completely destroying them as an edible fruit.

The disease is known to occur in South Carolina and has been reported from other southern States.

Ascospores of this fungus have been found on blooms which were collected not less than 8 feet above the nearest group of apothecia.

The occurrence of the microcondial form of the fungus in the diseased fruit is noted.

The most striking feature which distinguishes this fungus from others of the same group is the presence of a prominent caruncle-like body on the concave surface of the ascospore.



PLATE I

A.—Diseased fruits of *Morus alba*, showing the greatly enlarged condition of the ovaries.  $\times 1\frac{1}{3}$ .

B.—Photomicrograph of section of infected ovary, showing the sclerotium and layer of microconidia, indicated by arrow.  $\times 75$ .

C.—Infected ovaries (a), mass of microconidia on the outside.  $\times 3$

